Vasopressin in chronic kidney disease and its effects in autosomal dominant polycystic kidney disease

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Vasopressin in Chronic Kidney Disease and its Effects in Autosomal Dominant Polycystic Kidney Disease

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1

INTRODUCTION AND AIMS OF THIS THESIS

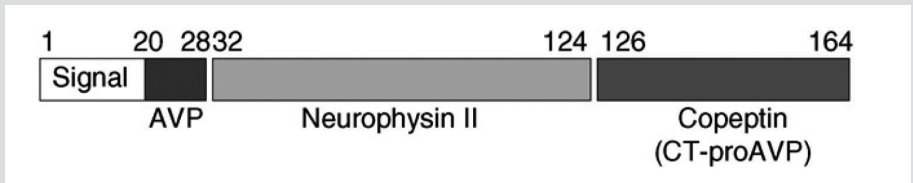
GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

Vasopressin

Vasopressin is also known as antidiuretic hormone. It is secreted by the hypophysis by stimulation of osmotic receptors in the hypothalamus when plasma osmolality increases, and by stimulation of baroreceptors in the carotid sinus in case blood pressure decreases. Vasopressin can bind to several receptors, the V1a, V1b and the V2 receptors. Binding to the V1a receptor leads to vasoconstriction, gluconeogenesis, platelet aggregation and release of factor VII and von Willebrand's factor. Binding to the V1b receptors leads to adrenocorticotropic hormone secretion. Most important, binding to the V2 receptors located at the basolateral side of collecting duct cells in the kidney leads to water reabsorption by the insertion of the water channel aquaporin-2 at the luminal side of these cells. Consequently, an increase in vasopressin leads to more concentrated urine and less concentrated plasma [1–3]. Vasopressin is therefore important to regulate the water balance.

Vasopressin is difficult to measure in human blood. It has a short ex-vivo half-life and it is partly bound to platelets [4]. The precursor that is released by the hypophysis (pre-pro-vasopressin) is split into fragments, among which vasopressin and copeptin (figure 1). Copeptin is the c-terminal part of the precursor and is therefore produced in same proportions as vasopressin. The function of copeptin is unknown, but in comparison with vasopressin, this protein is easier to measure, because it is not bound to platelets and stable in ex-vivo blood [5]. It is measured by chemiluminescence immunoassay and it has been shown that copeptin concentration has a strong correlation with vasopressin [6]. Copeptin concentration is therefore used as a marker for plasma vasopressin concentration.

Figure 1. Schematic of the precursor peptide of vasopressin [4]. Number indicates amino acids.



PART I THE ROLE OF VASOPRESSIN IN CHRONIC KIDNEY DISEASE PROGRESSION.

In experimental studies using chronic kidney disease (CKD) models, it was shown that high vasopressin levels lead to hypertension and glomerular hyperfiltration [7]. On the long term this leads to glomerulosclerosis, and albuminuria [8]. In these experimental models vasopressin receptor blockade by an antagonist led to less proteinuria and prevented the development of glomerulosclerosis and fibrosis. In humans, short-term infusion of vasopressin led to albuminuria [9]. In a cross-sectional general population based cohort, it was found that high copeptin levels are associated with higher albuminuria [10]. Higher copeptin levels were also associated with more rapid decline in kidney function in renal transplant recipients [11]. **Chapter 2** reviews the possible deleterious role of vasopressin in chronic kidney disease.

In humans it is known that vasopressin levels are higher in patients with diabetes mellitus compared with healthy subjects [12,13]. Moreover, a significant association was found between copeptin and the incidence of diabetes mellitus in a general population cohort [14]. In a cohort of diabetic patients with end stage renal disease copeptin was associated with cardiovascular mortality and all-cause mortality [15]. The question whether vasopressin is associated with a change in kidney function in chronic kidney disease, such as diabetes, was still not answered. In **chapter 3** we investigated the association between vasopressin, measured as copeptin, and change in kidney function (estimated GFR and albuminuria) in 1,328 patients with diabetes treated in a primary care setting. The goal of this study was to investigate whether vasopressin plays a role in kidney function loss in diabetes and thus may be a prognostic marker for kidney function decline in this patient group.

In **chapter 2** (review), as well as in this thesis, the special focus will be on the relation between vasopressin and autosomal dominant polycystic kidney disease (ADPKD). The reason for this focus is that vasopressin may play an important role in the development and growth of kidney cysts and kidney function loss in this disease.

Autosomal dominant polycystic kidney disease (ADPKD)

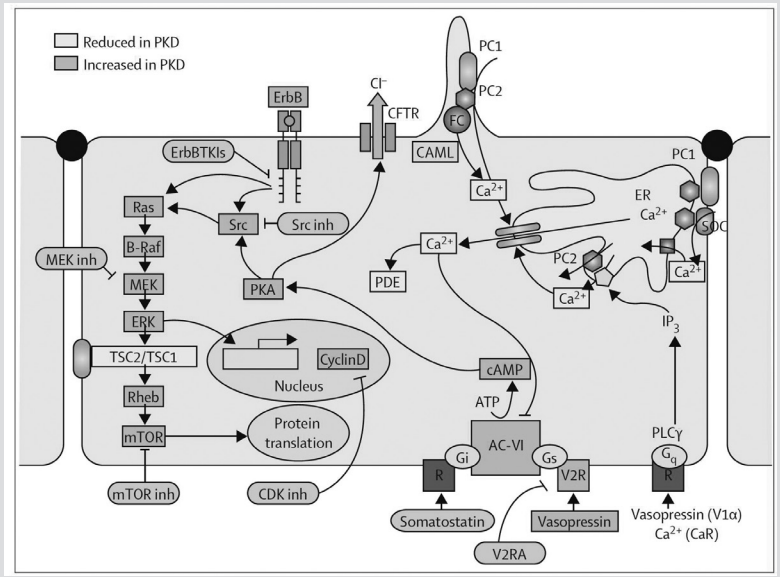
Autosomal dominant polycystic kidney disease is a genetic disease with an incidence of 1 per 400 to 1 per 1,000 [16]. It is characterized by cysts in the kidney, predominantly in the collecting ducts and distal tubules. Symptoms are kidney pain, hematuria, hypertension, and decreased kidney function. The most common extra-renal manifestations are liver cysts, heart valve abnormalities and cerebral aneurysms.

The renal cysts grow during life time and lead to enlargement of the kidneys. In an 'early' stage of the disease kidney volume increases, whereas kidney function can remain stable during prolonged periods of time [17]. However, the larger the kidneys, and the more cysts, the more likely damage of the kidney structure will develop, and the more likely that kidney function will decrease in the future. Therefore an increased kidney volume is an earlier marker for disease severity in ADPKD than kidney function [18].

ADPKD is caused by a mutation in the *PKD1* (85% of affected patients) [19] or *PKD2* (15% of affected patients) gene, located on chromosome 16 and 4, respectively [20]. This mutation results in a disturbance in the functionality of the polycystin proteins, that form a complex located in the cilia of the kidney collecting tubule cells. Due to this mutation, the dysfunctional polycystin complex does not respond adequately to flow stimuli, leading to a decrease in intracellular calcium, which normally inhibits the enzyme adenylylcyclase (AC) . Consequently, more activity of AC leads to an increase in intracellular cAMP, which in turn starts a chain of events, leading to cystogenesis by increased cell proliferation, abnormal fluid secretion and dedifferentiation. Binding of vasopressin to the V2 receptor, which is localized near AC, also leads to stimulation of AC, stimulating cell proliferation and fluid secretion leading to cyst formation and growth [16]. Part of this complicated pathway, in which vasopressin binding to V2 receptors leads to cyst growth, is shown in figure 2.

The growth of the kidneys and the decrease in kidney function differs between ADPKD patients with a *PKD1* or *PKD2* mutation. Patients with a *PKD1* mutation have a higher rate of kidney function loss and kidney volume growth than patients with a *PKD2* gene mutation [21]. The median age of start of renal replacement therapy in *PKD1* is between 40 and 60 years of age, and in *PKD2* between 60 and 80 years of age [16]. However, there is considerable variability between patients within families that share the same mutation [22]. The cause of this variability is not yet known, but vasopressin might play a role in the progression of disease severity in ADPKD as will be described in this thesis.

Figure 2. Hypothetical pathway up-regulated or down-regulated in ADPKD. Figure published in [16].



Cross-sectionally it was already found that higher copeptin levels (as surrogate for vasopressin) are associated with larger kidneys and lower kidney function in ADPKD. Because it is hypothesized that vasopressin stimulates cyst growth and therefore leads to a decrease in kidney function, we expected that vasopressin can also be used as a prognostic marker in these patients. In **chapter 4** copeptin was measured in a cohort of 79 ADPKD patients at baseline. These patients with a broad range of kidney function were followed over time and kidney function (GFR) was measured by a reference method (inulin clearance) during 3.3 years of follow-up and estimated by the MDRD equation during 11.2 years of follow-up. In this chapter the association between baseline copeptin and change in eGFR will be described. Also, the association between copeptin and start of renal replacement therapy was investigated. Because kidney size was not measured in this study, and GFR was estimated in the long-term study and not measured by a reference method, further research was needed to assess the value of copeptin as prognostic marker. In another cohort of 241 ADPKD patients we therefore studied the association between baseline copeptin and change in kidney volume (measured by MRI) and kidney function (measured as iothalamate clearance) during 8.5 years of follow-up (**chapter 5**). In this cohort only patients with relatively preserved kidney function were included, so we could investigate whether copeptin may be an early marker for disease progression in ADPKD and thus if vasopressin plays an important role in disease progression in an early stage of the disease.

PART II BLOCKADE OR STIMULATION OF VASOPRESSIN RECEPTORS IN ADPKD

In this part of the thesis we describe the effects of stimulation (by water deprivation) and blockade (by vasopressin V2 receptor antagonists) of vasopressin receptors in ADPKD.
ADPKD patients are less capable to concentrate their urine [23], probably due to the damage of the medullary structure by micro- and macro-cysts. Therefore their urine has a lower osmolality, and plasma osmolality will increase. As a feedback mechanism, vasopressin concentration will rise. This will result in a vicious circle, in which vasopressin levels become higher, which leading to more hyperfiltration and cyst growth and finally to more damage and less capacity to concentrate urine. In **chapter 6**, fifteen ADPKD patients with a normal kidney function and fifteen healthy age and gender matched controls participated in a water deprivation test. The goal of this study was to investigate whether a decreased urinary concentrating capacity exists already in early ADPKD, and what the mechanism might be. To investigate this, a water deprivation test was performed. We compared the maximum urine concentrating capacity, vasopressin and copeptin levels in ADPKD with controls. To exclude the possibility that ADPKD patients concentrate less by a lack of vasopressin release by the hypophysis, all participants received also a vasopressin analogue at the end of the test to see if they could further concentrate their urine with more (exogenous) vasopressin.

There is no treatment yet available to stop cyst growth and kidney function loss in ADPKD. However, several treatment options are developed at the moment, since some pathways of cyst formation are now known, as is shown in figure 2. One of the possible pathways is via the vasopressin V2-receptor. In **chapter 7**, the vasopressin V2 receptors were blocked by tolvaptan, a vasopressin V2 receptor antagonist. This drug was already tested in ADPKD patients with relatively preserved kidney function (creatinine clearance >60 mL/min). From this study it was concluded that tolvaptan slows the rate of cyst growth and kidney function decrease. In our study we investigated the renal hemodynamic effects in ADPKD patients with impaired kidney function compared with patients with normal and moderately impaired kidney function. This was investigated in a study in which we included 9 patients with an eGFR below 30 mL/min/1.73m^2 and compared them with 9 patients with a moderate kidney function (eGFR 30-60 mL/min/1.73m^2) and 9 patients with an eGFR >60 mL/min/1.73m^2. We measured GFR as iothalamate clearance before and during tolvaptan use and 3 weeks after withdrawal. We also measured various safety measures, such as blood pressure, plasma sodium, weight, and registered adverse events. All these measures were compared between the study groups to see whether tolvaptan had the same renal hemodynamic effects and was safe to use in ADPKD patients with impaired kidney function.

Besides the renal hemodynamic effects of tolvaptan, it is also important to know whether we can expect the same efficacy of tolvaptan in subjects with impaired kidney function. Long term efficacy can be measured by effects on GFR or total kidney volume, but these measures are quite expensive and labor-intensive. Therefore it would be interesting to find cheaper, easier to measure prognostic factors that predict outcome in a short period to predict for the long term. To that purpose, we measured in **chapter 8** urinary biomarkers reflecting damage to different parts of the nephron in patients with ADPKD, to see whether these markers are associated with GFR, effective renal plasma flow and kidney volume and thus with disease severity in ADPKD. This was a cross-sectional study in 102 ADPKD patients compared with 102 gender- and age-matched healthy controls. In **chapter 8b** we followed these ADPKD patients to see whether the urinary biomarkers measured in **chapter 8** were also predictive for change in eGFR during longer follow-up.
In the short-term study with tolvaptan in ADPKD patients described in **chapter 7**, we looked not only at renal hemodynamics and safety of tolvaptan, but we also investigated the short-term efficacy. In **chapter 9** various efficacy parameters were measured such as: kidney volume, GFR, urinary volume, free water clearance, and also most of the urinary biomarkers investigated in **chapter 8**. These variables were measured before the patients started to use tolvaptan, after 3 weeks of treatment with tolvaptan, and 3 weeks after the last dose, to investigate what the effects were of tolvaptan and whether potential effects were reversible. We evaluated whether the effects were associated with GFR at baseline, to document whether tolvaptan is as effective in ADPKD patients with lower GFR compared with ADPKD patients with normal GFR.

OVERALL AIM OF THIS THESIS

In this thesis the role of vasopressin on kidney function in CKD in general, and in ADPKD, particularly is studied in part I. The association between baseline copeptin concentration, a surrogate for vasopressin, and future kidney function decline was studied in diabetic patients and in ADPKD. In part II the renal effects of blocking or stimulating vasopressin in ADPKD subjects are studied. This thesis will give more insight in the effects of vasopressin on CKD in general, and on ADPKD particularly. This insight may hopefully help to better prediction of disease progression in CKD in the future, and lead to more and rational treatment options in ADPKD.

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PART 1

2

POTENTIAL DELETERIOUS EFFECTS OF VASOPRESSIN IN CHRONIC KIDNEY DISEASE AND PARTICULARLY AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Kidney Blood Press Res 34: 235–244, 2011

ABSTRACT

The antidiuretic hormone vasopressin is crucial for regulating free water clearance in normal physiology. However, it has also been hypothesized that vasopressin has deleterious effects on the kidney. Vasopressin is elevated in animals and patients with chronic kidney disease. Suppression of vasopressin activity reduces proteinuria, renal hypertrophy, glomerulosclerosis and tubulointerstitial fibrosis in animal models. The potential detrimental influence of vasopressin is probably mediated by its effects on mesangial cell proliferation, renin secretion, renal hemodynamics, and blood pressure. In this review, we discuss the increasing body of evidence pointing towards the contribution of vasopressin to chronic kidney disease progression in general and to autosomal dominant polycystic kidney disease in particular. These data allude to the possibility that interventions directed at lowering vasopressin activity, for example by the administration of vasopressin receptor antagonists or by drinking more water, may be beneficial in chronic kidney disease.

INTRODUCTION

Vasopressin is a nonapeptide hormone that is derived from a precursor synthesized in the hypothalamus. It is transported to the pituitary gland, where it is secreted in reaction to an increase in plasma osmolarity and a decrease in effective circulating volume [1]. Vasopressin is also synthesized in response to stress. There are three receptor subtypes known that can bind vasopressin, the V1a, V1b and V2 receptors. The V1a and V2 receptors mediate a number of different cellular effects, many of which are part of a response leading to water conservation. The V1a receptor is localized in smooth muscle cells, the liver and the kidney. In the kidney, the V1a receptor is mainly localized in the interlobular arteries, the descending vasa recta, the macula densa and the collecting duct [2]. Activation of the V1a receptor results in an increase in blood pressure by vasoconstriction. This increased vasoconstriction is the result of a direct effect on smooth muscle cells and of an indirect effect caused by increased renin secretion [3]. A complex interplay between V1a- and V2-receptor-mediated effects has been suggested [4, 5]. The V1b receptor is located in the adenohypophysis. Activation results in ACTH and subsequent cortisol release. This receptor is considered important for temperature regulation, memory and especially the stress response [6, 7]. The V2 receptor is found in the kidney and is predominantly localized in the collecting duct, but also in the macula densa and the thick ascending limb of Henle. Stimulation of the V2 receptor by vasopressin increases water reabsorption primarily by inserting aquaporin-2 water channels into the apical cell membrane of the collecting duct. Furthermore, it also increases urea reabsorption through the urea transporters UTA1 and UTA3. Sodium reabsorption is increased by a dose-dependent V2-receptor-mediated effect of vasopressin on the Na-K-Cl cotransporter in the thick ascending limb of Henle, on the Na-Cl cotransporter in the distal convoluted tubule [8] and, most importantly, on the epithelial sodium channel in the principal cells of the collecting duct [9]. Vasopressin V2 receptor activation thus not only increases free water permeability of the collecting duct, but also regulates medullary osmolarity, the driving force of water reabsorption. Subjects who lack vasopressin (central diabetes insipidus) or are insensitive to vasopressin (nephrogenic diabetes insipidus) have to drink large volumes of water to prevent dehydration, illustrating the physiologic importance of vasopressin.

POTENTIAL DELETERIOUS RENAL EFFECTS OF VASOPRESSIN

Mechanisms

Although vasopressin has a pivotal role in normal physiology, it has been hypothesized that in certain circumstances vasopressin may also have deleterious effects on the kidney. Vasopressin is described to negatively affect renal hemodynamics by increasing glomerular filtration rate (GFR) by two different mechanisms. Both mechanisms might

induce glomerular hyperfiltration on the short term. On the long term, this hyperfiltration will be detrimental. It has been postulated that intrarenal recycling of urea, triggered by vasopressin, might influence GFR adversely by modifying the composition of the tubular fluid at the macula densa, thus affecting the intensity of the tubuloglomerular feedback control of GFR [10-12]. Vasopressin could furthermore potentially stimulate the renin-angiotensin-aldosterone system directly because of the presence of V1a and V2 receptors in the macula densa.

Under physiological conditions, the renal vasculature and total renal blood flow are relatively insensitive to the action of vasopressin on the V1a receptor. However, in pathological conditions, a renal vasoconstrictor response is observed. In addition, vasopressin could theoretically induce hypertension by a direct effect on vascular smooth muscle through activation of the V1a receptor, or indirectly by V2 receptor-mediated increased tubular sodium retention [5, 13]. On the other hand, some have suggested that hypertension will be attenuated by vasodilatation due to prostaglandin release through V1a activation. Lastly, vasopressin has been suggested to exert direct effects on proliferation of mesangial cells and renal hypertrophy, production of collagen and fibronectin and inhibition of matrix metalloproteinase-2 [14, 15]. In summary, vasopressin may have deleterious effects on the kidney by causing increased glomerular pressure, renin release, hypertension and mesangial cell proliferation.

Experimental Evidence

Several observations in experimental studies are in line with a deleterious role for vasopressin in chronic kidney disease (CKD). First, spontaneous vasopressin levels have been found to be elevated in animal models with CKD [16, 17]. Second, administration of DDAVP (1-desamino-8- D -arginine vasopressin or desmopressin, a vasopressin V2 receptor agonist) increased proteinuria and worsened renal function after 5/6 nephrectomy in Brattleboro rats, that are characterized by a genetically determined vasopressin deficiency [18]. Deleterious effects were also observed in other models. Continuous administration of desmopressin to rats, resulting in an experimental SIADH (syndrome of inappropriate antidiuretic hormone secretion) model, induced renal histopathologic abnormalities, such as dilatation of tubules, and inflammatory cell infiltration, accompanied by significant increases in the relative weight of the kidney [19]. Desmopressin administration in uninephrectomized DOCA-salt hypertensive rats (a model for salt-dependent nonmalignant hypertension) led to worsening of hypertension and renal histology and an increase in albuminuria [20]. Third, inhibition of vasopressin activity ameliorates renal disease in CKD animal models. In an experiment in rats with 5/6 nephrectomy, vasopressin activity was suppressed by adding water to the chow of these animals, thus inducing a high water intake. After 5 weeks of high water intake, these rats had a lower percentage of glomeruli with segmental lesions than rats that received normal water intake [16]. In another study in rats with 5/6 nephrectomy, urinary volume increased and

urinary osmolarity decreased after the nephrectomy, suggesting impaired renal urinary concentrating capacity. Vasopressin levels were found to be elevated compared to control rats. When rats were forced to a high water intake, vasopressin levels decreased, and blood pressure, proteinuria and plasma creatinine were reduced [17]. When 5/6 nephrectomy is performed in genetically vasopressin-deficient Brattleboro rats, compensatory renal hypertrophy and CKD progression were attenuated when compared to rats that do secrete vasopressin [18]. Further evidence supporting a detrimental role for vasopressin in renal disease progression can be derived from experimental studies using vasopressin receptor antagonists. These studies are summarized in table 1, and are discussed in more detail in the section on vasopressin receptor antagonists.

Table 1: Experimental studies investigating a renal protective role of vasopressin receptor antagonists in chronic kidney disease (adapted from Torres et al (21)).

Study	Class of drug	Animal model	Results
Okada etal.(22), 1994	V1aRA, V2RA	DOCA-salt and adriamycin-treated rats	Both reduced blood pressure rise, the combination ameliorated histology
Okada et al.(23), 1995	V1aRA, V2RA	Uninephrectomized DOCA-salt hypertensive rats	V2RA and combination reduced blood pressure
Okada et al.(24), 1995	V1aRA, V2RA	5/6 nephrectomized SHR	Both reduced proteinuria, proteinuria and arteriosclerosis
Okada et al. (25), 1996	V1aRA, V2RA	Adriamycin-treated rats	Both reduced proteinuria and histologic alterations
Kurihara et al.(26), 1996	V1aRA	Uninephrectomized SHR	Reduced blood pressure, glomerular sclerosis and improved renal function
Naito et al.(19), 2001	V2RA	Sprague-Dawley rats	Prevented hypertrophy, tubular dilatation and interstitial infiltration
Bardoux et al.(27), 2003	V2RA	Streptozocin-induced diabetes mellitus	Prevented rise in albuminuria
Windt et al.(28), 2006	V1aRA	5/6 nephrectomized rats, starting 2 weeks after surgery	Reduced proteinuria and glomerular sclerosis
Windt et al. (28), 2006	V1aRA	5/6 nephrectomized rats, starting 6 weeks after surgery	No effect
Okada et al.(29), 2009	V2RA	Puromycin aminonucleoside nephrosis	Reduced proteinuria and kidney weight and improved renal function
Perico et al.(30), 2009	V1a/V2RA	5/6 nephrectomized rats	Reduced hypertension, proteinuria and glomerular sclerosis

Abbreviations are: V, vasopressin; RA, receptor antagonist, DOCA, deoxycorticosterone acetate; SHR, spontaneously hypertensive rats.

Human Evidence

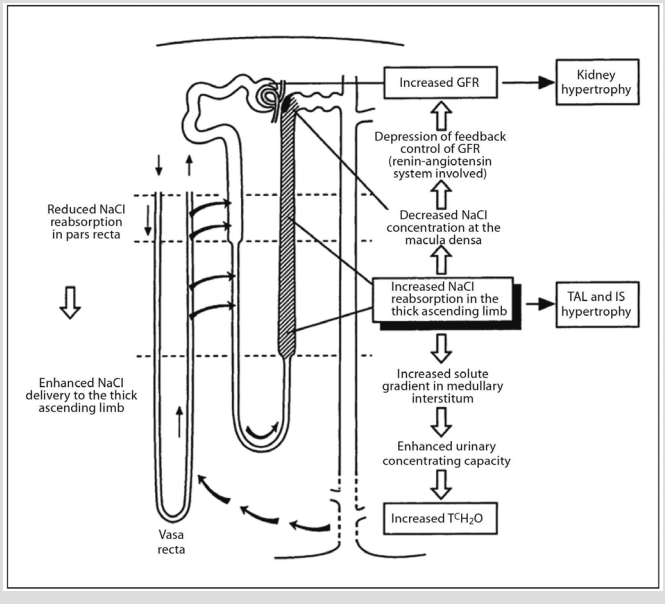
In humans, evidence for the potential deleterious role of vasopressin can be derived from observational data and from intervention studies inducing an increase or a decrease in vasopressin activity.

A retrospective analysis of data obtained in CKD patients participating in the MDRD study showed that higher urinary volume and lower estimated urinary osmolarity were associated with faster estimated GFR (eGFR) decline [31]. Two possible explanations for this relationship were offered. The first is that excessive fluid intake may cause faster renal disease progression. The other explanation is that, instead of the cause, a high

urinary volume with a low urinary osmolarity is merely the result of faster renal disease progression, with vasopressin thus as an innocent bystander. In our opinion, there may be a third option: a high vasopressin level may have caused faster renal disease progression. One of the clinical manifestations of CKD is a defect in urinary concentrating ability [32]. Because of this concentrating defect, higher vasopressin levels can be expected to compensate for the urinary concentrating defect. These higher vasopressin levels may in turn be causally related in renal disease progression, as suggested by the aforementioned animal experiments. Unfortunately, vasopressin concentration was not determined in the MDRD study. Definite conclusions on the role of vasopressin can therefore not be drawn upon these data.

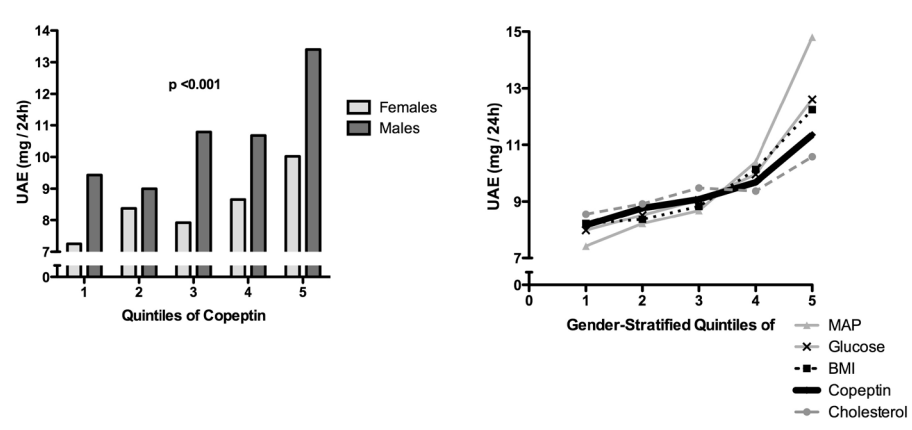
In general, studies in humans on the association between endogenous vasopressin concentration and onset or progression of CKD are scarce. This may at least partially be caused by the fact that direct measurement of vasopressin is problematic. More than 90% of vasopressin in the circulation is bound to platelets [33], vasopressin is unstable in isolated plasma [34], and most vasopressin assays have relatively limited sensitivity. Recently, an assay has been developed to measure copeptin, the stable C-terminal portion of the precursor of vasopressin [35]. Copeptin has been shown to be a reliable marker of vasopressin secretion and appears to be a clinically helpful method for indirectly assessing vasopressin plasma concentration [36]. For these reasons, in more recent clinical research, copeptin is measured as a surrogate for vasopressin concentration.

Figure 1: Potential detrimental influence of vasopressin on the kidney. Proposed sequence of events, illustrating how intrarenal recycling of urea (arrows), triggered by vasopressin and essential to the urinary concentrating mechanism, might influence GFR indirectly by modifying the composition of the tubular fluid at the macula densa and thus the intensity of the tubuloglomerular feedback control of GFR. This might induce glomerular hyperfiltration on the short term. On the long-term, this hyperfiltration will be detrimental. (Figure derived from Bankir and Kriz [10]).



In the PREVEND study, a large observational community-based population study, it was found that a high concentration of copeptin was associated with a low 24-hour urinary volume and a high 24-hour urinary osmolarity, consistent with normal physiology [37]. Furthermore, this study showed that copeptin concentration was positively associated with the amount of urinary albumin excretion (fig. 2a), even after adjustment for potential confounders. The association between albuminuria and copeptin was as strong as the association between albuminuria and known risk factors for albuminuria such as BMI, glucose and cholesterol (fig. 2b). In patients with diabetes mellitus, both vasopressin and copeptin were found to be elevated [38, 39] and to be inversely associated with eGFR (40). These cross-sectional observations are in line with the aforementioned experimental data showing that vasopressin promotes renal damage. Unfortunately, these studies did not investigate whether higher plasma copeptin levels predict eGFR loss.

Figure 2: Epidemiological evidence suggesting deleterious renal effects of vasopressin 7,593 subjects participating in a general population screening. Left panel. Copeptin concentration (as a surrogate for vasopressin) was positively associated with the amount of urinary albumin excretion. Right panel. This association is as strong as the association between albuminuria and known risk factors for albuminuria. [37]



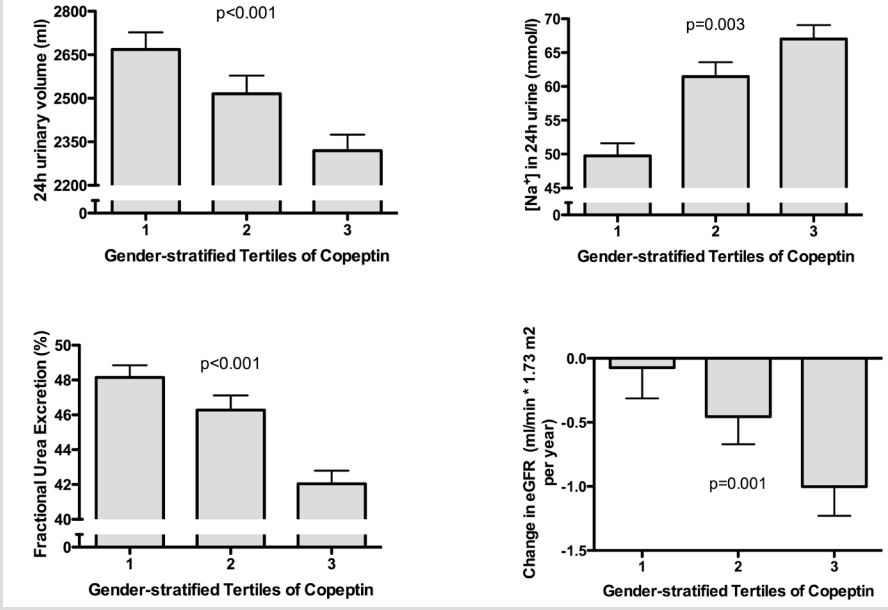
Abbreviations are: UAE, urinary albumin excretion; MAP, mean arterial pressure; BMI, body mass index.

This question was addressed in a study in 548 renal transplant recipients. It was shown that plasma concentration of copeptin predicted renal function loss over a 3.2-year follow-up period. This association was unmodified by adjustment for baseline eGFR, age, gender and other known risk factors for renal function decline in renal transplant recipients [41]. Similarly as in the PREVEND study, regulation and action of copeptin were consistent with normal physiology in this study, since at baseline a positive association between plasma osmolarity and copeptin concentration was found, as well as a negative association

between copeptin and 24-hour urinary volume and fractional urea excretion, and a positive association between copeptin and urinary sodium concentration as a surrogate for urinary concentrating capacity (fig. 3).

The most compelling evidence for a possible deleterious role of vasopressin in CKD can be derived from the pioneering experiments by Bankir et al. [42]. In 6 healthy volunteers, they showed that infusion of the vasopressin V2 receptor agonist desmopressin did not change creatinine and β2 -microglobulin excretion, but increased albuminuria markedly [42]. This suggests that the rise in albuminuria was due to an increased glomerular leakage of albumin. Desmopressin also increased albuminuria in patients with central diabetes insipidus and in patients with hereditary nephrogenic diabetes insipidus bearing aquaporin-2 mutations. However, albuminuria was not increased in patients with hereditary nephrogenic diabetes insipidus bearing mutations of the V2 receptor. As part of their experiment, rats were given ACE inhibitors and angiotensin-2 receptor blockers, which blunted the desmopressin-induced rise in albuminuria [42]. Given these data, the albuminuric effect of vasopressin seems to result from increased glomerular leakage, to require functional vasopressin V2 receptors, and to be mediated, at least in part, by the renin-angiotensin system.

Figure 3: Clinical evidence suggesting deleterious renal effects of vasopressin in renal transplant recipients (n=548). Copeptin plasma concentration (as a surrogate for vasopressin) is associated with 24-hour urinary volume (left upper panel), urinary sodium concentration (right upper panel) and fractional urea excretion at baseline (left lower panel), and predicts renal function decline during follow-up (median 3.2 years; right lower panel) [41].



Particularly Deleterious in Autosomal Dominant Polycystic Kidney Disease?

There is a relatively large body of experimental evidence suggesting that vasopressin may have a special deleterious role in the process of cyst growth and renal function decline in patients with autosomal dominant polycystic kidney disease (ADPKD). ADPKD is the most frequent hereditary kidney disease, characterized by cyst formation leading to kidney enlargement and renal failure. Activation of the vasopressin V2 receptor in renal collecting duct cells results in an increase in cAMP, which in the normal situation is inhibited by a calcium influx in the cell. In ADPKD, however, this process is not adequately inhibited. Mutations in the *PKD1* or *PKD2* gene cause a dysfunctional polycystin complex, inducing a decrease in intracellular calcium. As a result, intracellular cAMP rises, leading to cell proliferation and cyst fluid secretion, and consequently to cyst growth [43]. Furthermore, in animal models for ADPKD, vasopressin is elevated and V2 receptor expression upregulated [44, 45].

Figure 4: Clinical evidence suggesting deleterious renal effects of vasopressin in ADPKD patients (n=102, males = ▲ and -, Females = o and - - -). Copeptin plasma concentration (as surrogate for vasopressin) is associated with various markers of ADPKD severity [47]. Abbreviations are: TRV, total renal volume (left upper panel); UAE, urinary albumin excretion (left lower panel); ERBF, effective renal blood flow (right upper panel); GFR, glomerular filtration rate (right lower panel).

Subjects with ADPKD have a defect in urinary concentrating capacity. Gabow et al. [46] showed that this defect is already present early in the disease and parallels renal anatomical disruption by cysts: the more cysts, the more impaired the concentrating capacity. This suggests that ADPKD patients will have higher vasopressin levels to maintain fluid balance. Unfortunately, this study did not measure vasopressin or copeptin levels. This was done in a study we performed in 102 ADPKD patients at different stages of their disease [47]. Higher copeptin levels were associated with more albuminuria, larger kidneys, lower renal blood flow and reduced GFR (fig. 4). These associations were similar in males and females, and independent of potential confounders. In these ADPKD patients, plasma osmolarity was positively associated with copeptin, as expected. In contrast to the aforementioned studies in the general population [37] and renal transplant recipients [41], copeptin concentration was however not associated with 24-hour urinary volume, 24-hour urinary osmolarity or fractional urea excretion. This suggests that in ADPKD patients, vasopressin may not have the same physiologic consequences as in healthy subjects and renal transplant recipients.

Taking these data into consideration, we formulated a hypothesis of how vasopressin might be causally involved in the final common pathway of GFR decline in CKD in general, and in ADPKD in particular. In subjects with lower GFR, interstitial fibrosis due to loss of functioning nephrons will impair the normal medullary urea gradient. These subjects have therefore an impaired urinary concentrating capacity and consequently lower urinary osmolarity and higher urinary volume than subjects with normal renal function [48, 49]. Higher vasopressin levels will be necessary to maintain fluid balance. Patients with ADPKD have an additional anatomical disruption of the medullary architecture induced by cyst formation, which will also negatively affect the urea gradient and consequently diminish urinary concentrating capacity. According to this hypothesis, genetically determined progressive cyst formation will lead to impaired water reabsorption, in turn leading to higher vasopressin concentration. At the same time, these higher vasopressin levels will result in disease progression via mechanisms discussed in the section on the deleterious effects of vasopressin. These processes induce a vicious circle predisposing to further cyst growth, distortion of renal anatomy and renal function decline. Figure 5 depicts this hypothesis schematically.

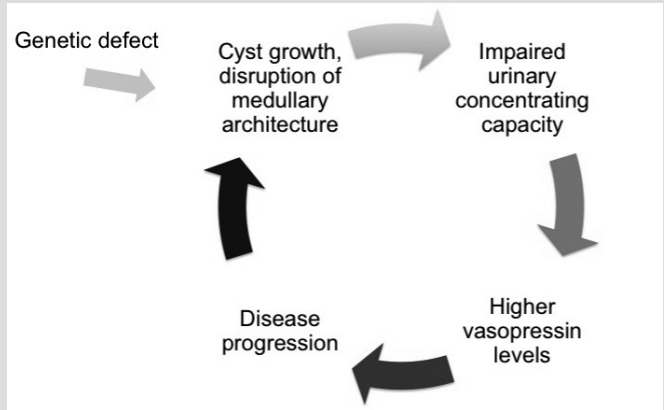


Figure 5: Schematic representation of the vicious circle by which vasopressin may lead to progressive renal function loss.

THE RENOPROTECTIVE POTENTIAL FOR VASOPRESSIN RECEPTOR ANTAGONISTS

In Chronic Kidney Disease

In the last two decades, several vasopressin antagonists have been developed. These drugs will provide the possibility to prove whether vasopressin is causally involved in renal disease progression. Several experimental studies have shown that V1a or V2 receptor antagonists, or both in combination, have a renoprotective effect in CKD (table 1). In a recent study, treatment with a dual V1a and V2 receptor antagonist, initiated 3 weeks after 5/6 nephrectomy, lowered blood pressure, proteinuria and glomerular sclerosis in Sprague-Dawley rats [50]. This effect was comparable to that of an ACE inhibitor or an angiotensin-2 receptor blocker. The combination of the vasopressin antagonist with an ACE inhibitor or angiotensin-2 receptor blocker, however, did not offer significantly more renoprotection. This suggests that part of the renoprotective effect of vasopressin antagonism is mediated by the renin-angiotensin system.

In ADPKD

Vasopressin V2 receptor antagonists have been shown to reduce cyst growth and preserve renal function in various animal models for polycystic kidney disease. These studies are summarized in table 2.

Table 2: Experimental studies investigating a renal protective role of vasopressin receptor antagonists in polycystic diseases.

Study	Class of drug	Animal model	Results
Gattone et al. (45), 2003	V2RA	PCK-rats (model for ARPKD)	Reduced renal cAMP levels, inhibited disease progression, reduced systolic blood pressure
Gattone et al. (45), 2003	V2RA	CD1/pcy mice (model for nephronophtisis)	Inhibited renal accumulation cAMP and decreased disease development
Torres et al.(44), 2004	V2RA	PKD2–/tm1Som mice (model for ADPKD2)	Reduced renal cAMP levels, prevented renal enlargement, inhibited cystogenesis, protected renal function
Wang et al. (51), 2005	V2RA	PCK-rats (model for ARPKD)	Reduced renal cAMP levels, fibrosis, and kidney weight
Meijer et al.(52), 2011	V2RA	PKD1 mice (model for ADPKD1)	Reduced cyst formation and kidney weight after 3 weeks administration; after 6 weeks effects were not significant anymore

Abbreviations are: V, vasopressin; RA, receptor antagonist, ARPKD, autosomal recessive polycystic kidney disease; ADPKD2, autosomal dominant polycystic kidney disease caused by a mutation in the PKD2 gene; ADPKD1, autosomal dominant polycystic kidney disease caused by a mutation in the PKD1 gene.

In human ADPKD, Torres et al. [53] analyzed data of a dose-ranging study monitoring long-term effects of the vasopressin V2 receptor antagonist tolvaptan. Forty-six patients were randomized 1:1 to open-label, low dose (45/15 mg) or medium dose (60/30 mg) of this drug. Their data suggest that the medium dose of tolvaptan during 3 years of follow-up slowed cyst growth and renal function deterioration when compared to the low dose. A large, phase 3, double-blind, placebo-controlled clinical trial is currently being conducted in patients with ADPKD with the same vasopressin V2 receptor antagonist, the TEMPO 3/4 study. This trial includes 1,445 patients with ADPKD who have relatively preserved renal function at baseline (estimated creatinine clearance ≥ 60 ml/min), but are anticipated to have progressive renal disease given the inclusion criterion of a large total kidney volume (≥ 750 ml). The primary outcome is the difference in growth rate in total kidney volume for tolvaptan compared to placebo. Secondary outcome parameters include time to multiple ADPKD progression events, such as time to a 33% change in serum creatinine or increase in albuminuria [54].

V1a versus V2 Blockade

The observation in animal models that blockade of both the V1a and the V2 receptor offers renoprotection suggests that stimulation of both receptors contributes to progression of CKD. The relative contributions of the V2 and V1a receptors, however, are ill defined. The dissection of V1a- and V2-dependent effects may become an important topic. Due to pharmacological blockade of the V2 receptor, a compensatory rise in vasopressin plasma levels is expected. In animal experiments, the rise in vasopressin is substantial [52], whereas in humans it has been described to be mild and within the physiological range. Whether this increase in vasopressin may have deleterious or beneficial effects on the unblocked V1a (and V1b) receptors is yet unknown and this question needs to be addressed in future

(clinical) studies. Of note, an effect on both receptors simultaneously can theoretically also be obtained by decreasing plasma vasopressin levels by means of increasing fluid intake. However, in order to achieve effective vasopressin suppression, fluid intake should be increased to obtain a urinary osmolarity of less than 300 mosmol/l. Dependent on sodium and protein intake, this implies increasing fluid intake to at least 4–6 liters per day. Whether adherence to such an advice is feasible for prolonged periods of time is questionable.

CONCLUSION

Vasopressin has a crucial role in water homeostasis by regulating free water clearance. Despite this essential role for normal physiology, an increasing body of evidence suggests that vasopressin also contributes to CKD progression. Plasma vasopressin levels are elevated in CKD animal models, as well as in patients with diabetic and nondiabetic nephropathies. Suppression of vasopressin activity ameliorates glomerulosclerosis and tubulointerstitial fibrosis in CKD models. Particularly in ADPKD, the most prevalent hereditary kidney disease, vasopressin is thought to have a pathophysiological role. The evidence for a detrimental role of vasopressin in CKD and the availability of vasopressin receptor antagonists allude to the possibility that these drugs may form a valuable addition to the current treatment armamentarium to attenuate renal function decline in patients with CKD in general, and in ADPKD in particular.

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COPEPTIN, A SURROGATE MARKER FOR ARGININE VASOPRESSIN, IS ASSOCIATED WITH DECLINING GLOMERULAR FILTRATION IN PATIENTS WITH DIABETES MELLITUS (ZODIAC-33)

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ABSTRACT

Aim/hypothesis. Arginine vasopressin (AVP), the hormone important for maintaining fluid balance, has been shown to cause kidney damage in rodent models of diabetes. We investigated the potential role of AVP in the natural course of kidney function decline in diabetes in an epidemiological study.

Methods. Plasma copeptin, a surrogate for AVP, was measured in baseline samples from patients with type 2 diabetes treated in primary care and included in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) cohort.

Results. Samples from 1,328 patients were available; 349 were analysed separately because they used renin–angiotensin–aldosterone system inhibition (RAASi), which influences albumin/creatinine ratio (ACR) and estimated (e)GFR. In the other 979 patients (46% men, age 68 years [58–75], ACR 1.8 mg/mmol [0.9–5.7], eGFR 67±14 ml min^{-1} 1.73 m^2) baseline copeptin (5.3 pmol/l [3.2–9.5]) was significantly associated with log_e[ACR] and eGFR, even after adjustment for sex, age and risk factors for kidney function decline (standardised [std] β 0.13, p<0.001, std β –0.20, p<0.001 respectively). Follow-up data were available for 756 patients (6.5 years [4.1–9.6]). Baseline copeptin was associated with increase in ACR (std β 0.09, p=0.02), but lost significance after adjustment (std β 0.07, p=0.08). Copeptin was associated with a decrease in eGFR after adjustment (std β –0.09, p=0.03). The strength of the association of copeptin with change in eGFR was stronger than that of established risk factors for kidney function decline (e.g. BMI, HbA_{1c}). In patients who used RAASi there was a significant association between baseline copeptin and ACR and eGFR, but not with change in ACR and eGFR.

Conclusions/interpretation. In patients with diabetes not using RAASi a higher baseline copeptin concentration is significantly associated with higher baseline ACR and lower eGFR values and with a decline in eGFR during follow-up. This last association is independent of, and stronger than, most traditional risk factors for kidney function decline.

INTRODUCTION

Arginine vasopressin (AVP), also known as antidiuretic hormone, plays an important role in the regulation of volume status. It is secreted into the blood on dehydration (increase in plasma osmolality) or volume loss [1]. The primary role of AVP is water reabsorption in the tubules by binding to the AVP V2 receptor [2]. In addition to this role in normal physiology, AVP has been hypothesised to have deleterious renal effects. In various experimental models, including rodent models of diabetes, it has been shown that AVP infusion induces hypertension, glomerular hyperfiltration, albuminuria and glomerulosclerosis [3-6]. In contrast, lowering AVP concentration by water loading resulted in less kidney damage [7].

It is known that AVP levels are higher in patients with diabetes compared with healthy individuals [8,9], especially in patients with diabetes and microalbuminuria [10]. Furthermore, it has been shown in humans that copeptin, a surrogate for AVP, is associated with an increased risk for diabetes [11,12] and that infusion of AVP increases albuminuria [13]. However, epidemiological studies investigating the association between AVP levels and the rate of kidney function decline are lacking.

The aim of the present study is to investigate the association between AVP (measured as copeptin) and the natural course of kidney function decline, cross-sectionally as well as longitudinally, in an observational cohort of patients with type 2 diabetes mellitus.

METHODS

Study sample and design

In 1998, the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study was initiated, as described in detail previously [14]. In the first year, 1,143 patients with type 2 diabetes mellitus participated. Briefly, the objective was to investigate the effects of a shared-care project for diabetes. Sixty-one general practitioners participated and were allocated to receive different degrees of support from diabetes specialist nurses for the practical implementation of the national guidelines in patients with known diabetes. The ZODIAC study was approved by the local medical ethics committee and all patients gave informed consent. In 2001 the ZODIAC cohort was extended to include 546 patients, resulting in a total of 1,689 patients. Plasma samples from 1,328 (79%) patients were available for the measurement of copeptin.

We divided the patients into two groups: patients who, at baseline, used medication that interferes with the renin–angiotensin–aldosterone system (RAAS), i.e. angiotensin converting enzyme (ACE) inhibitors and/or angiotensin receptor blockers (ARBs); and patients who did not use these medications. The analyses in patients who did not use RAAS inhibition (RAASi) at baseline are presented as primary analyses. This is because the aim of this study was to investigate the association between AVP (measured as copeptin) and the natural course of kidney function decline in type 2 diabetes, and RAASi is known to influence albuminuria and kidney function.

Data collection

At baseline, a full medical history was obtained, including medication use, diabetes duration and tobacco consumption. Physical and laboratory assessment included measurement of blood pressure (measured twice with a Welch Allyn sphygmomanometer in the supine position after at least 5 min of rest), weight, height, HbA_{1c} and non-fasting lipid profile. BMI was calculated from weight (kg)/height^2 (m).

AVP is difficult to measure in epidemiological studies because of platelet binding [15], a very short ex-vivo half-life [16] and a laborious assay. Copeptin is part of the precursor of AVP [17], and is more stable ex vivo and easier to measure (18). It has been shown to be a reliable surrogate for AVP [19]. Copeptin was measured in plasma samples collected at baseline and kept frozen at –80°C until analysis. It was measured by a chemiluminescence immunoassay (CT-proAVP LIA; Thermo Fisher Scientific, B.R.A.H.M.S. Biomarkers, Hennigsdorf/Berlin, Germany) as described previously [17], modified by replacing the capture antibody with a murine monoclonal antibody directed to amino acids 137–144 of copeptin. This modification improved the sensitivity of the assay. The lower limit of detection was 0.4 pmol/l [19].

Kidney function was assessed by measurement of (change in) albumin/creatinine ratio (ACR) and estimated (e)GFR. Serum creatinine was measured until March 2007 using a Jaffé method (Modular P Analyzer, Roche Diagnostics, Almere, the Netherlands), and thereafter by an enzymatic assay (Roche, Mannheim, Germany). A correction factor was applied to adjust enzymatic values. GFR was estimated using the modification of diet in renal disease (MDRD) equation [20], as advocated for creatinine measurements that are not isotope dilution mass spectrometry traceable. Urinary albumin concentration was measured using immunonephelometry (Behring Nephelometer; Mannheim, Germany). Serum creatinine and ACR were measured yearly if possible. Annual change in the log transformed (log_e) ACR and eGFR were calculated from the slope of the regression line through all available ACR and eGFR values during follow-up (provided that a minimum of three values were available). In cases where ACE inhibitor/ARB was started during follow-up, the last ACR or eGFR value before the start of this medication was used. This was because the aim of the present study was to investigate the association between copeptin and the natural course of kidney function decline, and the use of ACE inhibitors/ARB medication is known to impact progression in ACR as well as eGFR decline.

Statistical analyses

Analyses were performed with SPSS version 18.0 (SPSS, Chicago, IL, USA). Normal probability plots were inspected for deviances of normality. Continuous variables are expressed as mean (± SD) or as median (interquartile range [IQR]) for non-normally distributed variables. Variables with a skewed distribution were log transformed before analysis.

Before analysing the association between copeptin and ACR and eGFR, we tested interactions. The use or non-use of RAASi showed a significant interaction with plasma

copeptin level and eGFR at baseline (cross-sectional; std β=–0.21, p=0.003). There was a significant inverse association between copeptin and eGFR in the group not using RAASi and no significant association in the group that used RAASi. There were no significant interactions of RAASi use/non-use in the relationships between plasma copeptin and ACR, change in eGFR, or change in ACR. RAASi had a significant interaction with BP in all analyses (cross-sectional and prospective for ACR as well as eGFR). Therefore, all analyses were performed separately for patients with and without RAASi. We also tested for interaction between copeptin and sex. This interaction term was not significant in any of our analyses.

The associations between plasma copeptin and ACR and eGFR were analysed cross-sectionally and longitudinally by univariate regression analyses, with baseline copeptin value as the independent variable and (change in) ACR or eGFR as the dependent variable. Using linear multivariable regression analyses these associations were adjusted for covariates that could potentially be confounders in this association. First, crude analyses were performed (model 1). Second, multivariable models were built stepwise, entering possible confounders step by step. In model 2, the association was adjusted for sex, age and baseline ACR or eGFR (baseline ACR or eGFR only for the longitudinal analyses, baseline ACR for change in ACR and baseline eGFR for change in eGFR). Subsequently the association was adjusted for risk factors for progression of diabetic nephropathy (smoking, BP, HbA_{1c}, BMI, total cholesterol) and for duration of diabetes at baseline (model 3).

In the sensitivity analyses, changes in ACR and eGFR were calculated in a different manner: by subtracting the baseline from the last available ACR or eGFR and dividing by follow-up time (with a minimum follow-up of 1 year). Second, change in eGFR was calculated with GFR estimated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation (21). Third, we used linear mixed effects models to investigate the association between baseline copeptin concentration and ACR and eGFR over time. In addition to a crude analysis, we also performed analyses in which we adjusted for the covariates that were adjusted for in the main analyses (model 3).

For all analyses a two-sided p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

In the present study, 1,328 patients were included. Mean age was 66.8±11.6 years, 44.2% were men and the median duration of diabetes mellitus was 4 years (IQR 2–9). Baseline eGFR was inversely associated with baseline ACR. In the two groups, 349 patients used RAASi at baseline and 979 patients did not. Participant characteristics are shown in Table 1.

Table 1. Baseline characteristics, divided in the two populations: patients who did not use RAAS inhibition at baseline and patients who did.

	Non-RAASi	RAASi	p-value
N	979	349	
Male (%)	45.8	39.8	0.055
Age (yrs)	68	(58-75)	71	(60 – 77)	0.003
Weight (kg)	81	(71 – 91)	80	(73 – 94)	0.175
Systolic blood pressure (mmHg)	150.9	± 23.8	155.8	± 24.7	0.001
Antihypertensive drug use (yes, %)	30.1	100	<0.001		
HbA_{1c} (%)	7.1	(6.3 – 8.2)	6.9	(6.2 – 7.9)	0.011
HbA_{1c} (mmol/mol)	54.1	(45.4 – 66.1)	51.9	(44.3 – 62.8)	0.011
Diabetes therapy (%)					
Diet only	13.9		15.2		
Oral	67.1		66.8		0.792
Insulin	13.4		13.2		
Oral + insulin	5.6		4.9		
Cholesterol total (mmol/l)	5.6	(4.8 - 6.3)	5.4	(4.7 – 6.1)	0.039
Chol/HDL ratio	4.8	(3.9 – 6.0)	4.7	(3.9 – 5.8)	0.340
Lipid lowering medication (%)	11.5	22.5	<0.001		
Serum creatinine (µmol/l)	91	(81-102)	95	(85 – 109)	<0.001
BMI (kg/m^2)	28.3	(25.5 – 31.6)	29.4	(26.2 – 32.9)	0.003
Smoking (%)	20.7	14.6	0.013		
eGFR (MDRD, ml min^{-1} $(1.73m^2)^{-1}$	66.7	± 14.3	60.8	± 14.7	<0.001
eGFR CKD Stages:					
I. >90 ml min^{-1} $(1.73m^2)^{-1}$ (%)	6.0		2.0		
II. 60 - 90 ml min^{-1} $(1.73m^2)^{-1}$ (%)	61.4		48.7		
IIIa 45 - 60 ml min^{-1} $(1.73m^2)^{-1}$ (%)	27.3		36.7		<0.001
IIIb 30 - 45 ml min^{-1} $(1.73m^2)^{-1}$ (%)	4.7		10.6		
IV 15 - 30 ml min^{-1} $(1.73m^2)^{-1}$ (%)	0.6		2.0		
ACR (mg/mmol)	1.75	(0.89 – 5.71)	2.24	(0.97 – 7.15)	0.059
ACR CKD Stages:					
I <3.5 mg/mmol (%)	63.9		59.3		
II 3.5 – 35 mg/mmol (%)	28.5		29.5		0.321
III >35 mg/mmol (%)	4.9		6.6		
Duration of DM (years)	5.0	(2.0 – 9.0)	3.7	(1.8 – 9.0)	0.112
Copeptin (pmol/l)	5.3	(3.2 - 9.5)	5.7	(3.2 – 10.3)	0.174

Data are presented as mean ±SD or median (IQR), unless stated otherwise. C, cholesterol; CKD, chronic kidney disease.

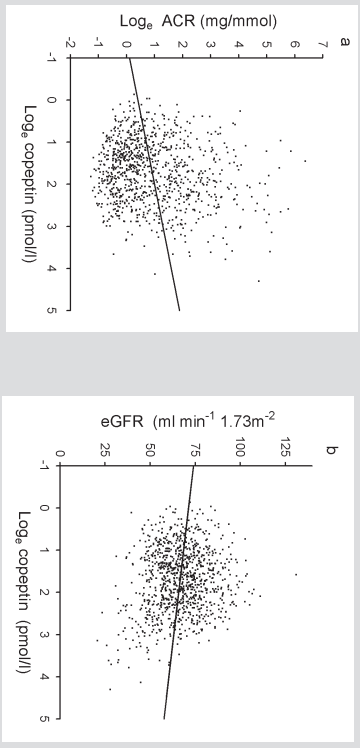


Figure 1. Baseline copeptin concentration and baseline albumin over creatinine ratio (ACR, left) and baseline eGFR (right) of subjects with diabetes not using RAAS inhibition at baseline.

Table 2. Associations between baseline copeptin concentration and baseline ACR and eGFR, and change in ACR and eGFR during follow-up in univariate (model 1) and multivariate models (models 2 and 3). Copeptin, HbA_{1c}, cholesterol, ACR, BMI and duration of DM were ln-transformed in these analyses of subjects with diabetes not using RAAS inhibition at baseline.

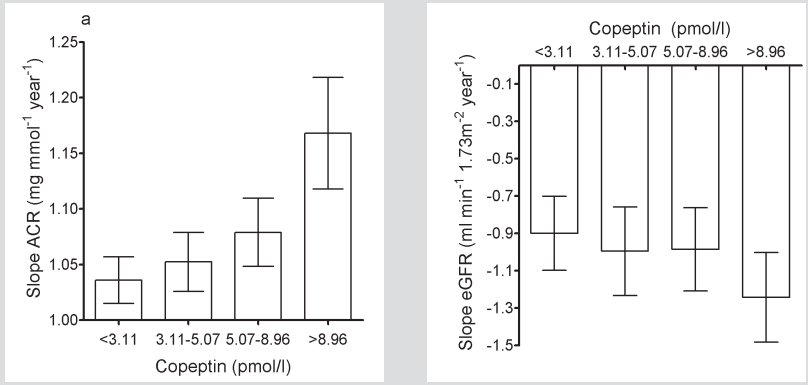
Ln ACR baseline N=955					eGFR baseline N=979				Change in ln ACR N=691				Change in eGFR N=756			
Model	Std β	b	95%CI	p	Std β	b	95%CI	p	Std β	b	95%CI	p	Std β	b	95%CI	p
1	0.162	0.297	0.18, 0.41	<0.001	-0.143	-2.727	-3.91, -1.54	<0.001	0.088	0.037	0.01, 0.07	0.020	-0.031	-0.131	-0.44, 0.18	0.402
2	0.157	0.287	0.17, 0.41	<0.001	-0.198	-3.777	-4.79, -2.76	<0.001	0.085	0.035	0.00, 0.07	0.034	-0.085	-0.363	-0.68, -0.04	0.027
3	0.133	0.243	0.13, 0.36	<0.001	-0.201	-3.856	-4.90, -2.81	<0.001	0.072	0.030	-0.00, 0.06	0.075	-0.087	-0.373	-0.71, -0.04	0.029

Model 1: crude
Model 2: as model 1 + age, gender, baseline eGFR (in change eGFR) or ACR (in change ACR)
Model 3: as model 2 + systolic blood pressure, cholesterol, HbA_{1c}, smoking, BMI, duration of diabetes

Table 3. Standardized betas of the multivariable model investigating the association between baseline copeptin and change in eGFR of subjects with diabetes not using RAAS inhibition at baseline. Copeptin, HbA_{1c}, cholesterol, ACR, BMI and duration of DM were ln-transformed in these analyses

	Standardized beta	95% confidence interval	p-value
Baseline eGFR	-0.315	-0.09, -0.05	<0.001
Copeptin	-0.087	-0.71, -0.04	0.029
Age	-0.081	-0.05, 0.00	0.071
Gender	-0.074	-0.99, 0.07	0.088
Duration of DM	-0.069	-0.34, 0.01	0.069
Systolic blood pressure	-0.066	-0.02, 0.00	0.088
HbA_{1c}	0.045	-1.01, 4.22	0.244
Cholesterol	0.031	-0.67, 1.64	0.412
BMI	-0.026	-1.94, 0.93	0.489
Smoking	0.019	-0.42, 0.70	0.615

Figure 2. Baseline copeptin concentration in quartiles and mean change in ACR (left) and eGFR (right) with standard error of the mean during follow-up of subjects with diabetes not using RAAS inhibition at baseline.



Cross-sectional analyses

ACR was measured at baseline in 955 patients not using RAASi (98% of the group). The median ACR was 1.75 mg/mmol (IQR 0.89–5.71). Baseline copeptin level was positively associated with $\log_e$ACR (Table 2 and Fig. 1a). When adjusted for confounders in the different models, this association remained similar (Table 2). The association remained significant when additionally adjusted for baseline eGFR (std β 0.12; p<0.001) or when additionally adjusted for use of antihypertensive drugs (std β 0.13; p<0.001).

Serum creatinine was measured at baseline in 979 patients (100%). The median creatinine level was 91 µmol/l (IQR 81–102) and mean eGFR (MDRD) was 66.7±14.3 ml min^{-1} 1.73 m^{-2}. Baseline plasma copeptin concentration and eGFR were inversely associated (Table 2 and Fig. 1b): patients with higher copeptin levels had lower eGFR (Fig. 1). This association remained similar after adjustment for age, sex and the aforementioned risk factors for renal function decline (Table 2). The association remained significant when additionally adjusted for baseline ACR (std β –0.20; p<0.001) or when additionally adjusted for use of antihypertensive drugs (std β –0.20; p<0.001).

Longitudinal analyses

In 691 patients (72%) ACR was available at baseline and at least two more measurements were made during a median follow-up of 5.5 years (IQR 3.2–7.8 years), with, on average, six ACR values per patient. Median change in ACR was 1.0 mg/mmol per year (0.9–1.2). Baseline plasma copeptin level was significantly associated with change in ACR (Fig. 2a). This association remained significant after adjustment for age, sex and baseline ACR (Table 2). This association lost significance after additional adjustment for risk factors for renal function decline (Table 2) and after further additional adjustment for baseline eGFR (std β 0.07; p=0.07). When additionally adjusted for use of antihypertensive drugs, the association remained the same (std β 0.07; p=0.08).

In 756 patients (77%) serum creatinine was available at baseline and at least two more measurements were made during a median follow-up of 6.5 years (IQR 4.1–9.6) with, on average, six creatinine values per patient. The median decline in eGFR was –1.0 ml min–1 1.73 m^{-2} per year (IQR –2.1–0.2). Baseline plasma copeptin level was associated with change in eGFR after adjustment for age, sex and baseline eGFR (Table 2 and Fig. 2b), and this association remained significant after adjustment for risk factors for renal function decline (model 3). We did not include ACR in the adjustments performed in the models listed in Table 2 because we did not consider ACR a potential confounder, but rather potentially in the causal pathway. However, further adjustment for baseline ACR did not materially change the association (std β –0.09; p=0.03). When additionally adjusted for use of antihypertensive drugs, the association remained significant (std β –0.09; p=0.03). The standardised (std) β values for the covariates in the fully adjusted linear regression model for change in eGFR are shown (Table 3).

The std β values of the association of baseline copeptin levels with change in eGFR were higher than the std β values of traditional risk factors for renal function decline, such as smoking, BMI, HbA_{1c} and total cholesterol.

Patients who used RAASi at baseline

In patients who used RAASi at baseline, median ACR was 2.24 mg/mmol (0.97–7.15) (n=333), mean eGFR was 60.8±14.7 ml min^{-1} 1.73 m^{-2} (n=349) and median copeptin concentration was 5.7 pmol/l (3.2–10.3) (Table 1). Copeptin was not significantly different between patients who used RAASi and patients who did not. The increase in ACR was 1.0 mg $mmol^{-1}$ $year^{-1}$ (0.8–1.1) and the median decrease in eGFR was –1.1 ml min^{-1} 1.73 m^{-2} $year^{-1}$ (–2.3–0.0). In these patients baseline copeptin level was significantly associated with baseline ACR and eGFR, but not with change in ACR or change in eGFR (electronic supplementary material [ESM] Table 1).

Sensitivity analyses

We performed the same linear regression analyses with change in ACR and eGFR calculated as baseline values subtracted from the last values available during follow-up divided by follow-up time. Essentially, similar results were obtained, though the associations were slightly less strong (fully adjusted model for change in ACR std β 0.09, p=0.10, and for change in eGFR std β –0.06, p=0.10). When we used the slope of GFR estimated by the *CKD-EPI* equation we also found essentially similar, but slightly less strong, results for cross-sectional as well as longitudinal analyses. In the fully adjusted models, the std β of the association between copeptin and baseline eGFR was –0.19 (p<0.001) and between copeptin and change in eGFR it was –0.06 (p=0.13).

When we used linear mixed-effect models, we found significant associations between baseline copeptin concentration and change in ACR and change in eGFR, in the crude as well as in the adjusted models (model 3: std β=0.142; 95% CI 0.05, 0.23; p=0.002 and std β=–0.918; 95% CI –1.70, –0.13; p=0.022, respectively).

Supplementary Table 1. Baseline characteristics of patients using RAAS inhibition at baseline.

N	349
Male (%)	39.8
Age (yrs)	71 (60 – 77)
Weight (kg)	80 (73 – 94)
Systolic blood pressure (mmHg)	155.8 ± 24.7
Antihypertensive drug use (yes, %)	100
HbA_{1c} (%)	6.9 (6.2 – 7.9)
Diabetes therapy (%) Diet only Oral Insulin Oral + insulin	 15.2 66.8 13.2 4.9
Cholesterol total (mmol/L)	5.5 ±1.1
Chol/HDL ratio	4.9±1.4
Lipid lowering medication (%)	22.5
Serum creatinine (umol/L)	95 (85 – 109)
BMI (kg/m²)	29.4 (26.2 – 32.9)
Smoking (%)	14.6
eGFR (MDRD, mL/min/1.73m²) Stages: I. >90 mL/min/1.73m² (%) II. 60 - 90 mL/min/1.73m² (%) IIIa 45 - 60 mL/min/1.73m² (%) IIIb 30 - 45 mL/min/1.73m² (%) IV 15 - 30 mL/min/1.73m² (%)	60.8 ± 14.7 2.0 48.7 36.7 10.6 2.0
ACR (mg/g) Stages: I <30 mg/g (%) II 30 – 300 mg/g (%) III >300 mg/g (%)	19.8 (8.5 – 63.2) 59.3 29.2 6.9
Duration of DM (years)	3.7 (1.8 – 9.0)
Copeptin (pmol/L)	5.7 (3.2 – 10.3)

Abbreviations are: ACEi, ACE-inhibition; ARB, angiotensin II receptor blocker; HbA_{1c}, glycated hemoglobin; HDL, high density lipoprotein; BMI, body mass index; eGFR, estimated glomerular filtration rate; ACR, albumin creatinine ratio; DM, diabetes mellitus

Supplementary Table 2. Associations between baseline copeptin concentration and baseline ACR and eGFR, and change in ACR and eGFR during follow-up in univariate (model 1) and multivariate models (models 2 and 3) in patients with who used RAAS inhibition at baseline. Copeptin, HbA_{1c}, cholesterol, ACR and BMI were log-transformed in these analyses.

	Baseline ACR N=333		Baseline eGFR N=349		Change in ACR N=274		Change in eGFR N=302	
Model	Std B	p	Std B	p	Std B	p	Std B	p
1	0.198	<0.001	-0.332	<0.001	0.051	0.398	-0.076	0.187
2	0.178	0.003	-0.369	<0.001	0.107	0.084	-0.062	0.338
3	0.214	<0.001	-0.370	<0.001	0.092	0.150	-0.062	0.358

Model 1: crude
Model 2: as model 1 + age, gender, baseline eGFR (in change eGFR) or ACR (in change ACR)
Model 3: as model 2 + cholesterol, HbA_{1c}, smoking, BMI, systolic blood pressure

Discussion

This study shows that baseline copeptin concentration is significantly associated with higher ACR, which is an early marker for renal damage, and with lower eGFR in patients with type 2 diabetes who were not using RAASi at baseline. Moreover, a higher baseline copeptin concentration was associated with a decline in eGFR during follow-up. The association with decrease in eGFR was significant after adjustment for sex, age, baseline eGFR and risk factors for renal function decline and appeared stronger than the association between traditional risk factors such as smoking, HbA_{1c} and cholesterol.

Diabetes is an important cause of end-stage renal disease (ESRD) worldwide [22]. Risk factors for ESRD, particularly those that are modifiable, are therefore important to recognise, especially in patients with diabetes. Copeptin has been found to be associated with albuminuria in the community [23, 24] and with rate of eGFR loss in other patient groups, such as those with autosomal dominant polycystic kidney disease and renal allograft recipients [25, 26]. The present data show that copeptin is also associated with lower kidney function at baseline and a decline in kidney function during follow-up in patients with type 2 diabetes. Therefore, it seems that copeptin is associated with kidney function decline in general and that the association of copeptin and kidney function is not specific for type 2 diabetes.

We found a significant association between baseline copeptin and change in ACR in our crude model as well as in the model adjusted for sex, age and baseline ACR. In the fully adjusted model this association was not significant. This difference in the strength of the association might reflect the smaller sample size for change in ACR compared with change in eGFR and the natural higher variability in ACR [27] than in eGFR, which makes it harder to find significant associations with this chronic kidney disease measure.

From the literature it is known that copeptin is associated with incident microalbuminuria in a general population cohort [23, 24] and with incident diabetes in the general population [11, 12]. Furthermore, it was recently shown that higher copeptin levels are associated with higher incidence of cardiovascular and all-cause mortality in patients with diabetes and ESRD [28]. Our results add information on the missing link in this chain of events, i.e. copeptin is also associated with renal function decline.

To summarise these conclusions, patients with high AVP levels early in life have a higher risk of developing diabetes mellitus [11], when they have diabetes they have a higher risk of developing higher levels of albuminuria and renal function decline, and when they reach ESRD, they have a higher mortality risk [28]. Given these data AVP seems to be an important factor in these patients. The pathophysiological mechanism by which AVP exerts these effects is yet not fully unravelled. With respect to the association between copeptin and change in eGFR, results obtained in rodent models of diabetes suggest that the underlying mechanism may be that AVP leads to hyperfiltration and then to albuminuria and glomerulosclerosis [6]. Other mechanisms have also been mentioned [29].

Interestingly, we found no significant association between copeptin and change in ACR or eGFR in patients with diabetes who were using RAASi at baseline. There may be at least two explanations for this observation. This group of patients was considerably smaller in size. Consequently, the lack of significant association might be a power problem, especially because we did find a significant association between copeptin and change in ACR when the two study groups were combined (with and without RAASi at baseline). Our finding may also indicate that the deleterious effect of AVP (measured as copeptin) is mediated at least in part via the renin–angiotensin system, which could also explain the significant interaction that we found between RAASi use and copeptin for the association with eGFR. Indeed, it has been suggested that high AVP levels stimulate RAAS, resulting in vasoconstriction and consequently higher systemic and glomerular BP [30]. Unfortunately, from the present dataset it is not possible to firmly conclude which of the explanations is most likely.

Some limitations of this study should be addressed. First, patients were allowed to eat and drink ad libitum at baseline when blood was drawn for copeptin assessment. AVP concentrations are influenced by water and osmolar intake and because intake was not standardised, copeptin concentration will be more variable. We expect that this would lead to effect dilution and therefore lead to an underestimation rather than an overestimation of the association between copeptin and (change in) ACR or eGFR. Second, fasting glucose levels were not measured. In patients with diabetes a high glucose level probably leads to an increase in plasma osmolality [31], and consequently to an increase in AVP [32]. Longstanding high glucose levels can cause renal function decline. It could therefore be that copeptin is not directly involved in the association between glucose regulation and renal outcome. However, when we adjusted for HbA_{1c}, the association of copeptin with change eGFR remained significant. Glucose regulation therefore seems less likely to explain the associations we found. We cannot be sure, though, that the effect of copeptin on glucose metabolism is not the reason for this association. Third, although the associations described were independent of possible confounders in our multivariate analysis, residual confounding cannot be excluded, as in any epidemiological study. We did not correct for multiple testing which may lead to less strong associations as our study is exploratory and the four endpoints (ACR, eGFR and changes in ACR and eGFR) are strongly interrelated.

The strengths of this study are that it included a relatively large observational cohort of patients with type 2 diabetes with a long follow-up. Both serum creatinine and ACR were available at baseline and, in most patients, during follow-up. Cross-sectional and longitudinal analyses of both outcome measures rendered generally similar results, i.e. a significant association with copeptin. These data combined suggest that our conclusion that copeptin is associated with renal outcome in patients with diabetes is valid.

Besides offering insight into a potential pathophysiological mechanism explaining the decline in kidney function in diabetes, our data may have clinical implications. This study suggests that copeptin might be a novel marker to predict kidney outcome in patients with diabetes. Furthermore, our findings suggest that it might be beneficial to lower AVP

levels in patients with diabetes. This can be done by increasing water intake, decreasing sodium intake and by blocking the receptors of AVP in the kidney, for instance by specific AVP V2 receptor antagonists. However, before such advice can be given, randomised controlled clinical trials should be performed studying whether these interventions, preferably in addition to RAASi, have a beneficial renal effect.

In conclusion, plasma copeptin concentration is significantly associated with kidney function decline in patients with type 2 diabetes not using RAASi. This result suggests that copeptin might be a new prognostic marker for kidney function decline in these patients and that it might be beneficial to lower AVP levels.

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DUALITY OF INTEREST

J. Struck is employed by Thermo Fisher Scientific, a company that manufactures and holds patent rights on the CT-pro-AVP assay. All other authors declare that there is no duality of interest associated with their contribution to this manuscript

CONTRIBUTION STATEMENT

HJGB, KHG and NK designed the ZODIAC study. HJGB, NK, ID, AA and JS collected data. WEB, IJR, KHG, SJLB, PEdJ and RTG analysed and interpreted data for this study. WEB drafted the manuscript. HJGB, NK, IJR, KHG, SJLB, ID, AA, JS, PEdJ and RTG reviewed and edited the manuscript critically. All authors gave final approval of this version to be published.

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4

COPEPTIN, A SURROGATE MARKER FOR VASOPRESSIN, IS ASSOCIATED WITH KIDNEY FUNCTION DECLINE IN SUBJECTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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ABSTRACT

Background. Experimental studies have suggested that vasopressin plays a detrimental role in autosomal dominant polycystic kidney disease (ADPKD). It is, however, unknown whether endogenous vasopressin concentration is associated with kidney function decline in subjects with ADPKD.

Methods. We measured plasma copeptin (a marker of vasopressin) in 79 ADPKD subjects with renal function assessed during short-term follow-up by inulin clearance measured glomerular filtration rate (mGFR) and during long-term follow-up by Modification of Diet in Renal Disease (MDRD) equation estimated GFR (eGFR).

Results. In these subjects (43% male, age 36.8 ± 10.1 years, GFR 96.8 ± 18.2 mL/min/1.73 m^2), median copeptin concentration at baseline was 2.71 [interquartile ranges (IQR) 1.63–5.46] pmol/L. Baseline copeptin concentration was inversely associated both with change in mGFR during follow-up for 3.3 (3.1–3.5) years, (R=-0.300, p = 0.01), as well as with change in eGFR during follow-up for 11.2 (4.5–14.3) years, (R =-0.302, p < 0.01). These associations were independent of age, gender and baseline GFR. Nine subjects started renal replacement therapy during follow-up of which eight had at baseline a copeptin concentration above the median in this population.

Conclusion. In ADPKD subjects, a higher copeptin concentration is associated with kidney function decline during follow-up, suggesting that copeptin may be a new marker to predict kidney outcome in ADPKD.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. It is characterized by the development of kidney cysts and progressive kidney function loss, often leading to end-stage renal disease [1, 2]. Mutations in two genes, *PKD1* and *PKD2* on chromosome 16 and 4, respectively, can lead to this disease. There is no proven treatment available yet, which can attenuate the rate of cyst formation and kidney function decline.

Vasopressin, also known as antidiuretic hormone, regulates water homeostasis in the body. It is secreted by the neurohypophysis in response to an increase in plasma osmolarity or a decrease in plasma volume. Vasopressin is known to bind to three receptors. The V_{1A} receptor is found in several organs, among others the kidney, where binding of vasopressin to this receptor results in a decrease in blood flow to the inner medulla and stimulation of prostaglandin synthesis [3]. The V_{1B} receptor stimulates the release of adrenocorticotropin from the anterior pituitary [4]. The V2 receptor is found in the kidney and is located at the interstitial side of the principal cells of the collecting ducts. Upon stimulation, these V2 receptors induce insertion of the water channel aquaporin-2 in the luminal membrane of the principal cells, which leads to reabsorption of water from the lumen of the collecting duct into the blood, thereby reducing water excretion [3].

In ADPKD, vasopressin is hypothesized to play a role in the pathogenesis of cyst formation in the kidneys. In experimental studies, it has been shown that when this hormone binds to the V2 receptors, production of cyclic adenosine monophosphate (cAMP) is stimulated in the principal cells [2, 5]. In turn, cAMP stimulates cyst production by promotion of fluid secretion and activation and proliferation of cyst-derived cells [6]. Furthermore, in animal models for ADPKD, it has been shown that blockade of the effect of vasopressin leads to a reduction of cyst formation [7-11].

Vasopressin is difficult to measure because of its binding to platelets [12], short half-life time [13] and instability in isolated plasma [14]. Copeptin is a part of the precursor of vasopressin (preprovasopressin) and can be measured by a recently developed sensitive sandwich immunoassay [15]. Copeptin has been shown to be stable in isolated plasma [16] and to be a reliable substitute for circulating vasopressin concentration [17].

Recently, we found an association between copeptin concentration and various markers of disease severity in subjects with ADPKD [18], supporting the results of the above-mentioned experimental studies that suggest vasopressin to have a deleterious effect in ADPKD. However, this study was cross-sectional in design, thus limiting firm conclusions on temporal relationships and a possible causal role for vasopressin in kidney function loss. In the present study, we therefore aimed to investigate prospectively the association between plasma copeptin concentration at baseline and the prognosis with respect to kidney function during follow-up in subjects with ADPKD. A priori, we hypothesized that copeptin concentration is associated with the rate of decline of kidney function in these subjects.

MATERIALS AND METHODS

Subjects

We included ADPKD subjects who participated in a trial investigating the efficacy of angiotensin-converting enzyme (ACE)-inhibition to preserve kidney function that was performed between 1994 and 1999 [19]. In this study, normotensive subjects were randomized to enalapril once daily (OD) (5 or 10 mg) or placebo. Hypertensive subjects were randomized to a stepup dosage regime with a maximum of 20 mg enalapril OD or 100 mg atenolol OD. Inclusion criteria for this trial were ADPKD as defined by the ultrasonographic criteria formulated by Ravine [20], age 18–70 years and plasma creatinine <2.5 mg/dL. Exclusion criteria were a history of myocardial infarction, cerebrovascular accident, presence of other kidney disease, diabetes mellitus, congestive heart failure, peripheral vascular disease, hepatic dysfunction, chronic use of immunosuppressants, NSAIDS, uricosurics and levodopa, adverse reactions to ACE inhibitors or pregnancy. This study found no beneficial effect of ACE inhibition; there was no difference in rate of kidney disease progression in both groups. Inclusion and exclusion criteria for the present study conform with those in the original trial. In addition, we excluded subjects with missing data.

Measurements and definitions

Baseline. At baseline, subjects visited an outpatient department and their medical history was taken. Blood pressure was measured three times, of which the average was taken. Body mass index (BMI) was calculated using the standard formula: weight (kilogram)/ square of height (metre). Blood was drawn at baseline while patients were fasting; they were allowed to drink water ad libitum. Plasma creatinine was measured using the Jaffe method. Total cholesterol and glucose concentrations were measured by standard methods. Plasma samples were stored at -80°C. All samples were collected between 1994 and 1996 and have been stored for a similar period of time. Morgenthaler et al. [15] showed that frozen storage did not have an effect on concentration of copeptin with recovery values of around 100%. Copeptin was measured in these samples by a sandwich immunoassay (CT-proAVP LIA; ThermoFisher Scientific, B.R.A.H.M.S. Biomarkers, Hennigsdorf/Berlin, Germany) as described previously [15, 21], with a modification insofar that the capture antibody was replaced by a murine monoclonal antibody directed to amino acids 137–144 of proAVP. This modification improved the sensitivity of the assay. The lower limit of detection was 0.4 pmol/L [22]. Copeptin measurements were carried out by an employee of B.R.A.H.M.S. (the manufacturer of the copeptin assay), who had no access to patient files and was therefore blinded for outcome.

Follow-up.

Kidney disease progression during follow-up was assessed in three different ways.

(i) Short-term follow-up: change in measured glomerular filtration rate (mGFR). At baseline and at the end of the 3-year study, GFR was measured after an overnight fast by inulin clearance. A loading dose of inulin was given in 10 min, followed by 3 h of continuous infusion. During the infusion, subjects stayed in the resting position and maintained hydration by oral water intake ad libitum. After 1.5 h, three urine samples were obtained over 30-min periods, with blood samples before and at the end of each collection period for determination of inulin concentrations. For each urine portion and corresponding blood sample, the mGFR was calculated and then averaged. mGFR was measured twice at the beginning of the study with a median time between the two measurements of 14 (7–26] days. The mean of the two measurements was used. mGFR was corrected for body surface area calculated by the Mosteller formula [23]. Change in mGFR was calculated as the difference between the baseline and last available mGFR value divided by follow-up time in years.

(ii) Long-term follow-up: change in estimated GFR (eGFR). Plasma creatinine was measured at baseline, and this value was used to estimate GFR using the abbreviated Modification of Diet in Renal Disease (MDRD) equation [24]. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was not used for our primary analysis because the creatinine values obtained at baseline were not standardized to IDMS-traceable values. Subjects were followed over time and the last available plasma creatinine value was obtained for assessment of the most recent eGFR. For subjects who started renal replacement therapy (RRT) during follow-up or who died, the last available creatinine value before the start date of RRT or death was used as the most recent value. The date at which creatinine was measured was used as end of follow-up. Change in eGFR was calculated as the difference between the baseline and last available eGFR value divided by follow-up time in years.

(iii) Long-term follow-up: incident RRT. If applicable, the start date of RRT was reported and we investigated the association between baseline plasma copeptin concentration and the hazard ratio for start of RRT. The hazard ratio should be interpreted as the increase of risk in case of a 10-fold higher copeptin level (due to the log transformation of copeptin).

STATISTICAL ANALYSES

Analyses were performed with SPSS version 18.0 (SPSS Inc., Chicago, IL). Normality was tested with the Kolmogorov–Smirnov test. Parametric variables are expressed as means with the standard deviation (±SD). Nonparametric variables are expressed as medians with interquartile ranges (IQR). A two-sided p-value of <0.05 was considered to indicate statistical significance. For the analyses of copeptin as predictor of change in renal function during follow-up, we performed continuous analyses by performing univariate regression analyses with baseline log copeptin as the independent variable and change in renal function (mGFR or eGFR) as the dependent variable. To visualize these associations, scatter plots were made and a regression line was drawn. Using a multivariable regression model, these associations were adjusted for covariates that could potentially be confounders in this association. We built multivariable models stepwise. Firstly, our association was adjusted for gender and age (Model 2), and subsequently also for baseline GFR, use of diuretics, hypertension (defined as systolic blood pressure >140 or diastolic blood pressure >90 or use of anti-hypertensive drugs) and treatment group (placebo/atenolol versus enalapril) (Model 3). We adjusted for use of diuretics because these drugs influence volume status, and therefore vasopressin concentration. To investigate the association between plasma copeptin and incident RRT (Analysis 3), Cox regression analysis was performed. This analysis was performed similarly, first crude and subsequently stepwise with adjustment for gender, age, baseline eGFR, use of diuretics, hypertension (defined as systolic blood pressure >140 or diastolic blood pressure >90 or use of anti-hypertensive drugs) and treatment group (placebo/atenolol versus enalapril). Subjects were censored at the date they deceased, lost to follow-up, started RRT or at the date of the last available serum creatinine value. For regression analyses, logarithmic transformation (Lg10) of copeptin was applied to fulfil the requirement of equal distribution of the residuals. Interactions between log copeptin concentration and age and gender were tested for change in mGFR, eGFR and start of RRT as dependent variables.

RESULTS

Of 108 available subjects, 29 subjects were excluded because of missing plasma samples at baseline or missing data during follow-up (Figure 1). Baseline characteristics of the remaining 79 subjects are given (Table 1). Fortythree per cent of these subjects were male and mean age was 36.8 ± 10.1 years. Median baseline plasma copeptin concentration was 2.71 (1.63–5.46) pmol/L. The 29 subjects that were excluded for the present analyses did not differ significantly from the 79 subjects that were included with respect to any of the characteristics listed in Table 1, except for baseline GFR (mGFR 71.4 ± 32.5 mL/min/1.73 m² versus 96.8 ± 18.2 mL/min/1.73 m², respectively, p < 0.01; eGFR 59.4 ± 22.7 mL/min/1.73 m²

Figure 1. Flow diagram with in/exclusion of subjects for the three analyses: baseline plasma copeptin concentration versus 1. change in mGFR (inulin clearance) during short-term follow-up, 2. change in eGFR (MDRD) during long-term follow-up and 3. start of RRT during long-term follow-up. Abbreviations are: mGFR, measured glomerular filtration rate, eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy.

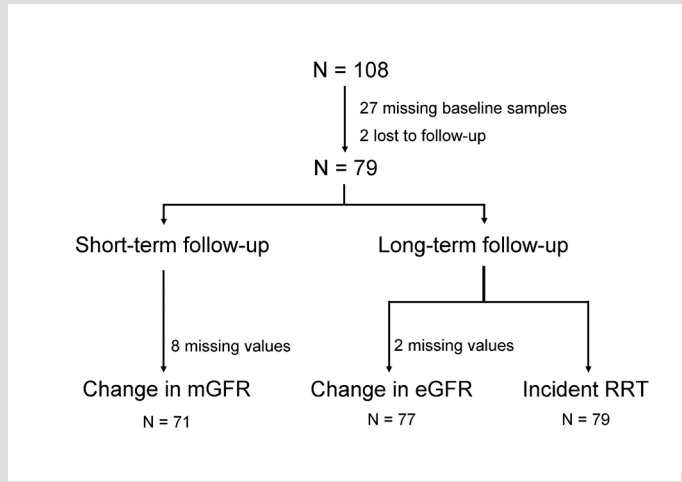

Table 1. Baseline characteristics of all 79 subjects analyzed.

Variables	
Male gender, n (%)	34 (43)
Age (years)	36.8 ± 10.1
BMI (kg/m²)	24.4 (21.9 – 26.2)
Systolic blood pressure (mmHg)	136.5 ± 16.4
Diastolic blood pressure (mmHg)	86.5 ± 8.5
Serum glucose (mg/dL)	85 (81 – 92)
Serum total cholesterol (mg/dL)	196 ±34
Serum creatinine (mg/dL)	1.0 (0.87 – 1.9)
mGFR (by inulin clearance in ml/min/1.73m²)	96.8 ± 18.2
eGFR (by MDRD in ml/min/1.73m²)	75.9 ± 18.1
Copeptin (pmol/l)	2.71 (1.63 – 5.46)

Results are given as means ± SD or as median (IQR) in case of non-normal distribution.
Abbreviations are: BMI, body mass index; mGFR, measured glomerular filtration rate, eGFR, estimated glomerular filtration rate. Conversion factors for units: serum gloucose in mg/dL to mmol/L, x 0.05551, serum total cholesterol in mg/dL to mmol/L, x 0.02586 and serum creatinine in mg/dL to µmol/L, x 88.4.

versus 75.9 ± 18.1 mL/min/1.73 m², respectively, p < 0.01) and systolic blood pressure (145.0 ± 16.7 mmHg versus 136.6 ± 16.4 mmHg, respectively, p = 0.03). In these 79 patients, 43 patients were randomized to enalapril, 5 to atenolol and 31 to placebo. The limited size of the subgroup receiving atenolol did not allow formal analyses of the efficacy of atenolol versus enalapril.

Baseline associations

Baseline mGFR and baseline eGFR were significantly associated with each other (std B 0.776, P < 0.001). Plasma copeptin concentrations were inversely associated with mGFR (std B-0.258, P = 0.02) and with eGFR (std B -0.207, p = 0.06). Age was not associated with plasma copeptin concentration (std B 0.052, p = 0.64).

Short-term follow-up: change in mGFR

For the first analysis of the association between copeptin and kidney outcome in ADPKD, inulin clearances assessed at baseline and at the end of the clinical trial in which the subjects under analysis participated were used. At the beginning of the trial, mGFR was assessed twice, with a coefficient of variation of 4.9%. In eight subjects, mGFR was not assessed at the end of the trial. These subjects were therefore excluded from this analysis, leaving 71 subjects, who had a median follow-up of 3.3 (3.1–3.5) years with a mean change in mGFR of -2.5 ± 3.7 mL/min/1.73 m²/year (Table 2). Baseline copeptin was significantly associated with change in mGFR during the trial (std B-0.300, p = 0.01) (Figure 2). When adjusted for gender, age, use of diuretics, baseline mGFR, hypertension and treatment group, a significant association remained between baseline plasma copeptin concentration and change in mGFR during short-term follow-up (std B-0.345, p < 0.01, Table 3).

Figure 2. Baseline plasma copeptin concentration versus change in mGFR (inulin clearance) during short-term follow-up [n = 71, follow-up 3.3 (IQR 3.1-3.5) years, standardized B -0.300, P = 0.01]. Triangles represent patients who started RRT during follow-up.

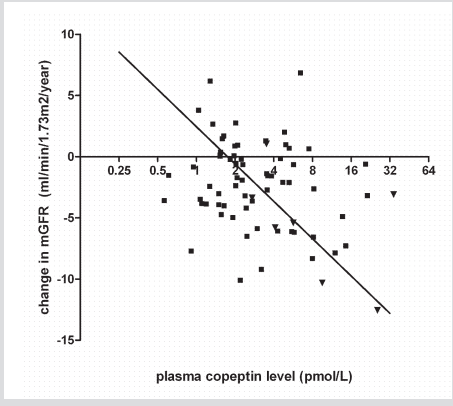

Table 2. Results during short- and long-term follow-up, where mGFR is measured as inulin clearance (Analysis 1) and eGFR is estimated by the MDRD equation (Analyses 2)[a].

Variables	mGFR (inulin clearance)	eGFR (MDRD)
N	71	77
Baseline GFR (ml/min/1.73m²)	97.1 ± 19.2	75.4 ± 18.1
Follow-up (years)	3.3 (3.1 – 3.5)	11.2 (4.5 – 14.3)
GFR end of study (ml/min/1.73m²)	90.0 ± 23.3	57.7 ± 29.5
Annual change in GFR (ml/min/1.73m²/year)	-2.5 ± 3.7	-1.7 ± 1.8
Died (all cause), n (%)		4 (5.2)
Start of RRT, n (%)		9 (11.7)

[a] Results are given as means ± SD, or as median (IQR) in case of non-normal distribution.
Abbreviations are: mGFR, measured glomerular filtration rate, eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy

Long-term follow-up: change in eGFR

For the second analysis, the baseline and last available creatinine values were used to estimate GFR with the abbreviated MDRD equation [24]. Two subjects were lost to follow-up, leaving 77 subjects for analysis, who had a median follow-up of 11.2 (4.5–14.3) years with a mean change in eGFR during long-term follow-up of -1.7 ±1.8 mL/min/1.73 m²/year (Table 2). Baseline plasma copeptin concentration was significantly associated with the decline in eGFR during long-term follow-up (std B-0.302, p < 0.01, Figure 3). Results of the multivariable regression analyses for this end point are shown (Table 3). When adjusted for gender, age, use of diuretics, baseline eGFR, hypertension and treatment group, the significant association remained between baseline copeptin concentration and change in eGFR during long-term follow-up (std B-0.254, p = 0.05).

Table 3. Association between baseline plasma copeptin concentration and change in mGFR (n= 71), change in eGFR (n = 77) and start of RRT (n = 79) during follow-up (3.3 and 11.2 years, respectively)[a].

	Model 1		Model 2		Model 3	
	Std B	p-value	Std B	p-value	Std B	p-value
Change in mGFR (inulin clearance)						
Lg10[Copeptin] (pmol/L)	-0.300	0.01	-0.341	0.004	-0.345	0.006
Gender (female)			-0.242	0.04	-0.222	0.07
Age (year)			-0.244	0.03	-0.175	0.2
Baseline mGFR (mL/min/1.73m²)					0.073	0.6
Use of diuretics (yes)					NA	NA
Hypertension (yes)					-0.180	0.1
Treatment group (enalapril)					-0.087	0.5
Change in eGFR (MDRD)						
Lg10[Copeptin] (pmol/L)	-0.302	0.008	-0.272	0.02	-0.254	0.05
Gender (female)			0.114	0.3	0.158	0.2
Age (year)			-0.007	0.9	0.090	0.6
Baseline eGFR (mL/min/1.73m²)					0.105	0.6
Use of diuretics (yes)					0.098	0.4
Hypertension (yes)					-0.141	0.2
Treatment group (enalapril)					-0.137	0.3
Incidence RRT	**HR**	**p-value**	**HR**	**p-value**	**HR**	**p-value**
Lg10[Copeptin] (pmol/L)	9.88	0.01	10.20	0.02	5.73	0.1
Gender (female)			0.45	0.3	0.02	0.02
Age (year)			1.02	0.5	0.99	0.9
Baseline eGFR (mL/min/1.73m²)					0.81	0.02
Use of diuretics (yes)					0.00	0.9
Hypertension (yes)					0.12	0.2
Treatment group (enalapril)					15.75	0.09

[a] Abbreviations are: mGFR, measured GFR; eGFR, estimated GFR; RRT, renal replacement therapy, aHR, hazard ratio, Std B, standardized beta, NA, not applicable (because none of the patients included in this analysis used diuretics), Lg10[copeptin], 10log transformed value of copeptin was used. The hazard ratio should be interpreted as the risk increase with a tenfold higher copeptin level.

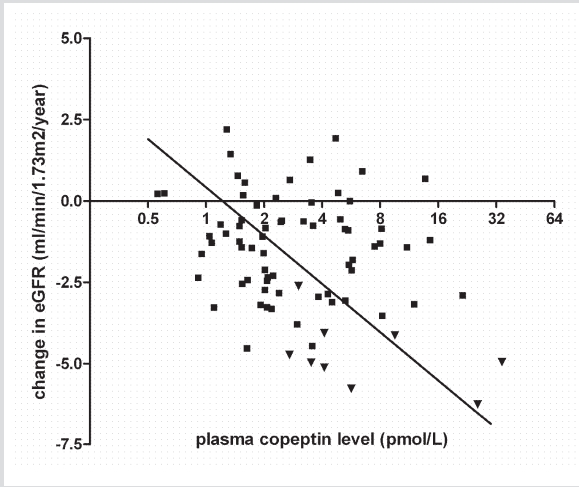

Figure 3. Baseline plasma copeptin concentration versus change in eGFR (MDRD) during long-term follow-up [n=77, follow-up 11.2 (IQR 4.5-14.3) years, standardized B -0.302, p<0.01]. Triangles represent patients who started RRT during follow-up.

Long-term follow-up: incident RRT

For the third analysis, the association between baseline copeptin concentration and start of RRT during follow-up was investigated. Of the 79 subjects with long-term follow-up, 4 subjects died, whereas 9 subjects started RRT (11.4%). Eight of those 9 patients had a copeptin concentration above the median value of 2.71 pmol/L. Subjects who started RRT were compared to subjects who did not start RRT for all characteristics listed in Table 1. Those who started RRT had similar characteristics as those who did not, except for plasma copeptin concentration [4.10 (3.27–17.6) versus 2.27 (1.55–5.19) pmol/L, p = 0.01], BMI [22.6 (19.9–24.0) versus 24.7 (22.1–26.2) kg/m^2, p = 0.03] and eGFR (60.0 ± 19.9 versus 77.5 ± 16.9 mL/min/1.73 m^2, p = 0.03). Results of the Cox regression analyses are given (Table 3). In the crude analyses, a 10-fold higher plasma copeptin concentration was associated with a hazard ratio for start of RRT of 9.88 (95% confidence interval: 1.72–56.88, p = 0.01). When adjusted for age and gender, the hazard ratio was 10.20 and remained significant (p = 0.02). When additionally adjusted for baseline eGFR, use of diuretics, hypertension and treatment group, the hazard ratio for incident RRT was lowered and of borderline statistical significance.

Sensitivity analyses

Several sensitivity analyses were performed. Firstly, we investigated in the short-term follow-up study the association between baseline copeptin concentration and change in eGFR (MDRD) instead of mGFR: crude std B-0.305 (p = 0.01) and Model 3 std B-0.259 (p = 0.07). Secondly, we used in the long-term follow-up study the CKD-EPI equation instead of the MDRD equation to estimate GFR: crude std B-0.312 (p < 0.01) and Model 3 std B-0.261, p = 0.05. Thirdly, change in the reciprocal of serum creatinine concentration over time was used as outcome measure for the short-term follow-up study crude std B-0.325 (p =

0.007) and Model 3 std B-0.285 (p = 0.05) as well as the long-term follow-up study crude std B-0.335 (p = 0.003) and Model 3 std B-0.298 (p = 0.02). All these analyses showed essentially similar results when compared with our primary analyses. In addition, we tested whether there was interaction between baseline copeptin and age or gender in the association with risk. No significant interactions were found for all the three analyses.

DISCUSSION

This study shows that in subjects with ADPKD, plasma concentration of copeptin is associated with a decline in kidney function, assessed as either change in inulin clearance (mGFR) during short-term follow-up or as change in eGFR during long-term follow-up. These associations remained significant after adjustment for age, gender, baseline GFR, diuretics, hypertension and treatment group. Furthermore, plasma concentration of copeptin was found to be associated with need for RRT during long-term follow-up, independent of age and sex.

As far as we know, the present study is the first to prospectively assess the association between baseline copeptin concentrations and renal outcome in subjects with ADPKD. Interestingly, we previously found a cross-sectional association between copeptin concentrations and disease severity in ADPKD [18], copeptin concentration and albuminuria in healthy subjects [25] and also an association between copeptin and accelerated renal function decline during follow-up in kidney transplant recipients [26]. Both studies showed a positive association, the higher copeptin the worse renal outcome. These studies, in combination with the present one, suggest that copeptin is associated with renal outcome in ADPKD, but possibly also in other chronic kidney diseases. From other studies, it is known that copeptin values can decrease very rapidly [15] suggesting extrarenal clearance as predominant clearance mechanism. Nevertheless, we took the possibility into account that lower renal function may lead to less clearance of copeptin and adjusted therefore for baseline GFR in our multivariable models. This adjustment led to only a minor decrease in the regression coefficient of the association between baseline copeptin and renal outcome. Consequently, this association remained significant. Furthermore, we recently found that under standardized conditions, copeptin levels were significantly elevated in young ADPKD patients when compared to age- and sex-matched healthy controls. Importantly, these young ADPKD patients and healthy controls appeared to have similar kidney function (eGFR CKD-EPI 100 versus 104 mL/min/1.73 m^2 and 24 h creatinine clearance 116 versus 117 mL/min/1.73m^2, respectively) [27]. These data suggest that in ADPKD patients, the association between copeptin level and renal outcome is unlikely to be an effect of a lower renal clearance of copeptin at baseline, and indicate that a rise in copeptin precedes kidney function decline. We propose that relatively early in the course of ADPKD, due to a urinary concentrating defect, vasopressin levels are elevated in order to maintain plasma

osmolality within the normal range. Unfortunately, plasma osmolality was not measured at the start of this study.

Our results also agree with the possible detrimental role of vasopressin in ADPKD, since the rise in vasopressin (measured as copeptin) precedes a decline in GFR. Consequently, it may be hypothesized that lowering vasopressin can lead to renoprotection in human ADPKD. To lower vasopressin concentration, one of the options is to achieve ample hydration. Another way to suppress the effect of vasopressin is to block the V2 receptor in the kidney with medication. This option has been tested in animal experiments, which showed that vasopressin antagonism indeed prevented cyst growth and kidney function decline [9]. At this moment, a large-scale randomized controlled trial is ongoing that investigates whether these vasopressin V2 receptor antagonists are renoprotective in ADPKD patients [28].

Our data suggest that plasma copeptin concentration may be a promising new, relatively easy to measure marker to predict kidney function decline in subjects with ADPKD. Nowadays, it is difficult to predict the prognosis of a patient with ADPKD. There are risk factors known, such as the type of genetic mutation (*PKD1* or *PKD2*) and family history with respect to age at time of need for RRT. Both are, however, not very specific at an individual level [29] and the genetic mutation is relatively difficult and time-consuming to measure for routine diagnostics. The same holds true for renal blood flow [30] and magnetic resonance imaging-assessed kidney (or cyst) volume [31]. Measurement of GFR is of limited value because it remains for a prolonged period near normal [32]. Plasma copeptin concentration may therefore be a new prognostic factor to help distinguish between patients with a low risk of reaching end-stage kidney disease and patients with a higher risk, alone or in combination with other prognostic factors. Replication of our findings is, however, necessary before it can be used as such.

Some limitations of this study should be addressed. Firstly, not all subjects who participated in the original study were included in the present analyses. The reason to exclude these subjects was, however, due to a random process (missing plasma samples) and no bias is therefore to be expected. Secondly, kidney volume was not assessed as outcome because at the time of initiation of this study, kidney volume measurements were not assessed routinely, nor in the context of clinical trials. Thirdly, baseline creatinine measurements were performed in one centre and standardized. During follow-up, however, the most recent creatinine was measured at various sites. Therefore, differences between assays for creatinine may be present. However, this is expected to result in effect dilution bias and therefore in an under- rather than an overestimation of the associations that were found. Fourthly, baseline copeptin concentration in these patients was measured only once. However, this is again expected to result in an under- rather than overestimation of the true effect size. Fifthly, only a limited number of incident RRT cases were observed during follow-up. The results of the analyses studying this outcome should therefore be interpreted with caution, and be regarded primarily as supporting the

findings that were obtained in the analyses studying the association between baseline copeptin and changes in mGFR and eGFR. Lastly, from this study, it cannot be concluded that copeptin predicts outcome because of a pathophysiological role of vasopressin in ADPKD specifically or that it is a marker for kidney disease progression in general.

Strengths of this study are that this study has a relatively long duration of follow-up and that renal outcome was measured in three different ways, among which the gold standard for measuring GFR, being inulin clearance. Our findings, showing that baseline copeptin concentrations are associated with all three renal outcome measures during follow-up, make our data robust and likely to be valid.

In conclusion, plasma copeptin concentration, a surrogate marker for vasopressin, is associated with the rate of kidney function decline in subjects with ADPKD. These results suggest that in such subjects, copeptin may be a promising, relatively easy to measure new marker to predict renal outcome, alone or in combination with other markers.

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CONFLICT OF INTEREST STATEMENT

J.S. is an employee of ThermoFisher Scientific, B.R.A.H.M.S. Biomarkers, the company that manufactures and holds patent rights on the copeptin assay. None of the other authors have anything to declare.

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RELATIONSHIP OF COPEPTIN, A SURROGATE MARKER FOR ARGININE VASOPRESSIN, WITH CHANGE IN TOTAL KIDNEY VOLUME AND GFR DECLINE IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE: RESULTS FROM THE CRISP COHORT

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ABSTRACT

Background: Experimental studies indicate that arginine vasopressin (AVP) may have deleterious effects in the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD). However, the significance of AVP in human ADPKD is unclear.
Study Design: Longitudinal observational study with 8.5 (IQR, 7.7-9.0) years' follow-up (CRISP [Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease]).
Setting & Participants: 241 patients with ADPKD with creatinine clearance >70 mL/min.
Predictor: Plasma copeptin concentration, a surrogate marker for AVP.
Outcomes: Change in measured glomerular filtration rate (mGFR, assessed by iothalamate clearance) and total kidney volume (measured by magnetic resonance imaging).
Measurements: Baseline copeptin level, plasma and urinary osmolality, and measurements of total kidney volume and mGFR during follow-up.
Results: In these patients (median age, 34 [IQR, 25-40] years; 38% men; median mGFR, 94 [IQR, 79-145] mL/min/1.73 m^2; median total kidney volume, 859 [IQR, 577-1,299] mL), median copeptin level was 2.9 (IQR, 1.8-5.1) pmol/L. Copeptin was not associated with plasma osmolality (P = 0.3), the physiologic stimulus for AVP release, but was associated significantly with change in total kidney volume during follow-up (P < 0.001). This association remained significant after adjusting for sex, age, cardiovascular risk factors, and diuretic use (P = 0.03). Copeptin level was associated borderline significantly with change in mGFR after adjusting for these variables (P = 0.09).
Limitations: No standardization of hydration status at time of copeptin measurement.
Conclusions: These data show that in ADPKD, copeptin level, as a marker for AVP, is not correlated with plasma osmolality. Most importantly, high copeptin levels are associated independently with disease progression in early ADPKD. This is in line with experimental studies that indicate a disease-promoting role for AVP.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease; its estimated prevalence is about 1 in 1,000. This disease is characterized by progressive bilateral cyst formation in the kidneys, which leads to pain, hematuria, and end-stage kidney failure, usually occurring in the age range of 30-60 years [1]. To date, there is no proven effective treatment to delay disease progression.

Arginine vasopressin (AVP) is hypothesized to have an important role in the pathogenesis of ADPKD. When AVP is bound to the V2 receptor at the basolateral side of the thick ascending limb and collecting duct epithelial cells, cyclic adenosine monophosphate (cAMP) production is stimulated. In turn, cAMP leads to the proliferation of epithelial cells and chloridedriven fluid secretion into cysts, thus leading to cyst formation and cyst growth [2,3].

Despite AVP's having a suspected key function in the pathophysiology of ADPKD, little is known about whether AVP is related to disease progression in patients with ADPKD. One of the reasons for this paucity of data might be that measurement of AVP is problematic because it is unstable ex vivo [4]. Therefore, we measured copeptin, the carboxy-terminal portion of the precursor of AVP[5], which has been shown to be reliable and stable and a valuable surrogate of circulating AVP concentration [6,7]. Previous studies have shown a strong association between AVP and copeptin, with a rapid decrease in copeptin level when AVP level is decreasing.

We recently found that plasma copeptin concentration was associated with various measures of disease severity in a cross-sectional study of 102 patients with ADPKD (8). Furthermore, we found an association between baseline copeptin concentration and rate of kidney function decrease in another cohort of 79 patients with ADPKD [9]. Limitations of this latter study are the relatively small sample size, limited information about covariates for adjusted analyses, and the fact that only glomerular filtration rate (GFR) was measured and not total kidney volume, the other kidney outcome measure used to assess disease progression in patients with ADPKD.

Therefore, we aimed in the present study to investigate the association between plasma copeptin concentration and kidney disease progression, measured as decrease in measured GFR (mGFR) and increase in total kidney volume, in a large well-phenotyped cohort of patients with ADPKD. To achieve this aim, data from the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) cohort [10] were used. A priori, we hypothesized that plasma copeptin levels at baseline are associated with accelerated kidney function decrease and increase in total kidney volume.

METHODS

Study Population and Study Design

The CRISP Study is a multicenter observational cohort study of patients with ADPKD that was created to develop imaging techniques and analyses to follow up disease progression and evaluate treatment for ADPKD. Detailed descriptions of the study protocol have been published elsewhere [10–14]. In summary, patients were included at 4 clinical sites: Mayo Clinic College of Medicine in Rochester, MN; University of Alabama at Birmingham; Emory University in Atlanta, GA; and the Kansas University Medical Center in Kansas City. Washington University in St Louis during CRISP I and the University of Pittsburgh during CRISP II served as the data coordinating and image analysis center. Patients with ADPKD (Ravine criteria [15]) in a relatively early phase of disease were eligible. Inclusion criteria therefore were set at age older than 15 and younger than 46 years and measured or estimated creatinine clearance >70 mL/min. Patients were ineligible when they had undergone kidney surgery or cyst drainage procedures, were unable to undergo breath hold magnetic resonance imaging (MRI), or had other medical conditions besides hypertension that potentially could affect kidney function (eg, diabetes mellitus).

After signing an informed written consent, enrolled patients were scheduled for a 2-day evaluation in the General Clinical Research Center at baseline and years 1, 2, and 3. Approximately 2 years after completion of CRISP I, participants were contacted and after informed written consent, enrolled individuals were scheduled for a baseline CRISP II visit (year 6). After 2 years, magnetic resonance measurement of total kidney volume and iothalamate clearance were performed again (year 8). Before each visit, participants were instructed to continue their medications, discontinue any nonsteroidal anti-inflammatory medications for at least 7 days before evaluation, and not initiate diuretic therapy within 14 days of evaluation. Weight, height, and body mass index were measured at admission. Blood pressure was measured in the morning before antihypertensive medication intake in the left and right arms after being seated for at least 5 minutes on 3 occasions 3 minutes apart using an oscillometric measuring device. Blood and spot urine samples were collected in the morning prior to hydration for the mGFR studies or taking medications or food. Plasma electrolytes, creatinine, serum urea nitrogen, lipid profiles, osmolality, and glucose were measured in these blood samples using standard laboratory techniques. Urine osmolality was measured in these spot urine samples by freezing point depression. In addition, plasma samples were stored at -80°C for later assessment of potential biomarkers predicting disease progression.

Measurement of Copeptin

Copeptin was measured in these samples, which were transported in frozen condition to the laboratory. Morgenthaler et al [5] showed that frozen storage did not have an effect on copeptin concentration, with recovery values of ~100%.Asandwich immunoluminometric

assay (CT-proAVP LIA BRAHMS; Thermo Scientific), which was based on the assay described previously [5], was used for assessment. It was modified so that the capture antibody was replaced by a murine monoclonal antibody directed to amino acids 137-144 of pro-AVP. This modification improved the sensitivity of the assay. The lower detection limit was 0.4 pmol/L and the functional assay sensitivity (20% interassay coefficient of variation) was <1 pmol [16]. Blood samples were collected in the morning prior to hydration for the mGFR studies or taking medications or food.

Measurement of GFR

Kidney function was measured by iothalamate clearance as described previously [13]. Briefly, after oral hydration, patients received a subcutaneous injection of nonradiolabeled iothalamate. After a 60-minute equilibrium period, each patient voided and the first plasma sample was drawn. After a timed 45- to 60-minute collection period to determine urine flow (V), a voided urine sample and second plasma sample were obtained. Postvoid residuals were assessed by ultrasound after each void. The 2 plasma (P) samples and 1 urine (U) sample were assayed for iothalamate through capillary electrophoresis at the Mayo Clinic. Iothalamate concentrations in plasma samples were averaged, and mGFR was determined using the clearance equation ($U_{iothalamate}$ x $V/P_{iothalamate}$). The mean between-site coefficient of variation for mGFR was 4.9% [13].

Measurement of Total Kidney Volume

Total kidney volume was measured by MRI performed in the morning before medication intake and breakfast [10–14]. Coronal T2-weighted images (single-shot fast spin-echo/half-Fourier acquired single-shot turbo spin-echo) and gadolinium-enhanced 3-dimensional volume interpolated spoiled-gradient echo coronal T1-weighted images were obtained (3-mm slice thickness). The data coordinating and image analysis center collected and analyzed images. Volumes of individual kidneys were measured in T1-weighted images with a stereology method and calculated from the set of contiguous images by summing the products of the area measurements and slice thickness. The reliability coefficient was 0.998 for total kidney volume in repeatedly acquired images for individual patients. The average coefficient of variation of total kidney volume measurements in the repeated analysis of 99 images was 0.01%. During CRISP II, gadolinium-enhanced T1-weighted images were no longer obtained because of concerns raised in 2006 about the role of gadolinium in nephrogenic systemic fibrosis [17]. In addition to T2-weighted imaging, a fast imaging sequence, 2-dimensional true-fast imaging with steady state precession (FISP) T2/T1-weighted imaging of the kidneys without fat saturation, was obtained to help delineate the kidney borders. The image analyst displayed these images concurrently as a visual guide and performed kidney volume measurement on T1-weighted images using the stereology method just as in the CRISP I image analysis.

STATISTICAL ANALYSES

Analyses were performed with SPSS, version 18.0 (SPSS Inc). Normality of data distribution was tested with the Kolmogorov-Smirnov test. Variables are expressed as median with interquartile range (IQR). Baseline characteristics are shown in sex-stratified tertiles of copeptin levels and differences between these groups were tested with the Kruskal-Wallis test.

Spearman correlation analysis was performed to investigate whether plasma copeptin concentration at baseline was correlated with physiologic variables and variables representing disease severity.

For analyses of copeptin as predictor of change in kidney measurements, individual patient annual change in mGFR (in mL/min/1.73 m² per year) was determined, and change in total kidney volume was calculated as the slope of ln[total kidney volume] using regression analysis, taking into account all data points available during follow-up. First univariate linear regression analyses were performed with baseline copeptin level as independent variable and change in mGFR or total kidney volume as dependent variable. Subsequently, these associations were adjusted for covariates that potentially could be confounders in this association using multivariable regression models. Multivariable models were built stepwise. The associations between plasma copeptin level and change in mGFR and total kidney volume during follow-up were adjusted for sex and age (model 1). Additionally, associations were adjusted for covariates that are causally linked to GFR loss in chronic kidney disease in general [18] or in ADPKD in particular [14] (body mass index, mean arterial pressure, and high-density lipoprotein cholesterol level; model 2), and additionally adjusted for a covariate that may be a confounder in the association between copeptin levels and outcome measures (use of diuretics; model 3). Last, we tested in the full adjusted model (model 3) the effect of additional adjustment for baseline mGFR when analyzing change in mGFR and of additional adjustment for baseline total kidney volume when analyzing change in total kidney volume. There was no regression to the mean when we looked at the change in total kidney volume or mGFR compared with baseline total kidney volume or mGFR; therefore, adjusting for baseline values was not needed. However, we added these extra analyses as a fourth model. For all regression analyses, logarithmic transformation of all variables without normal distribution was applied to fulfill the requirement of equal distribution of the residuals.

Because average copeptin values were different between men and women, we used for Figs 1 and 2 sex-stratified tertiles of copeptin, meaning that for men and women, tertiles were prepared separately and combined. Thus, each tertile in Figs 1 and 2 contains the same number of men and same number of women. These sex-specific tertiles had the following cutoff points for copeptin: first tertile, men <2.57 pmol/L and women <1.90 pmol/L; second tertile, men, 2.57-4.88 pmol/L and women, 1.90- 3.81 pmol/L; and third tertile, men >4.88 pmol/L and women >3.81 pmol/L, respectively.

Table 1. Baseline characteristics per sex-stratified tertile of plasma copeptin.

	Copeptin			
	Low (N=75)	Medium (N=74)	High (N=76)	p
Male sex	39	38	38	0.9
Age (years)	33.7 (26.3-39.1)	33.3 (24.5-39.9)	34.4 (24.3-40.2)	0.9
Smoking	16	19	13	0.6
BMI (kg/m²)	24.7 (21.4-29.1)	24.7 (21.8-27.5)	25.9 (22.2-29.7)	0.4
MAP (mmHg)	91.4 (84.3-98.5)	94.0 (85.8-99.8)	94.8 (84.7-102.8)	0.2
- Use of any antihypertensives	49.3	57.5	61.8	0.3
- Use of diuretics	12.0	9.6	10.5	0.9
Serum creatinine (mg/dL)	0.9 (0.8-1.1)	0.9 (0.8-1.1)	1.0 (0.8-1.2)	0.09
SUN (mg/dL)	14 (12-15)	13 (11-16)	16 (13-19)	<0.001
Serum sodium (mEq/L)	138 (136-140)	139 (137-140)	138 (137-139)	0.7
Serum osmolality (mOsm/L)	281 (278-284)	282 (279-285)	281 (279-284)	0.6
Serum glucose (mg/dL)	87 (80-94)	90 (83-96)	89 (84-97)	0.07
Serum HDL (mg/dL)	46 (38-60)	48 (41-54)	45 (36-55)	0.4
Urine volume (mL/24hr)	2533 (1926-3222)	2300 (1578-3243)	2158 (1450-2909)	0.2
Spot morning urine osmolality (mOsm/kg)	275 (158-412)	239 (167-451)	438 (329-531)	<0.001
GFR (mL/min/1.73m²)	100.5 (88.7-117.8)	98.5 (78.2-121.4)	85.9 (72.8-102.4)	<0.001
Total kidney volume (mL)	778 (532-1110)	777 (587-1219)	1098 (640-1609)	0.02
Copeptin (pmol/L)	1.5 (1.1-1.8)	2.9 (2.3-3.5)	6.4 (5.0-8.9)	<0.001

Note: Values for categorical variables are given in percent; values for continuous variables are given as median (interquartile range). Differences between groups were tested by the Kruskal-Wallis test. Conversion factors for units: serum creatinine in mg/dL to µmol/L, x88.4; SUN in mg/dL to mmol/L, x0.357; serum glucose in mg/dL to mmol/L, x0.05551; plasma HDL-C in mg/dL to mmol/L, x0.02586. Abbreviations: BMI, body mass index; MAP, mean arterial pressure; SUN, serum urea nitrogen; HDL-C, high-density lipoprotein cholesterol; mGFR, measured glomerular filtration rate (corrected for body surface area).

Table 2. Spearman rank correlation between plasma copeptin concentration and physiologic variables and measures of disease severity

	R	p
Plasma osmolality (in mOsm/L)	0.071	0.3
Urine volume (in mL/24 hr)	0.004	0.9
Morning urine osmolality (in mOsm/L)	0.312	<0.001
Mean arterial pressure (in mm Hg)	0.146	0.03
GFR (in mL/min/1.73m²)	-0.286	<0.001
Total kidney volume (in mL)	0.172	0.01

Note: N=225. Abbreviation: GFR: glomerular filtration rate (corrected for body surface area).

Interactions between copeptin concentration and age and sex were tested in the crude and full adjusted model with change in mGFR and change in total kidney volume as dependent variables. As a sensitivity analysis, the mentioned associations were studied in mixed models instead of linear regression analyses. Last, it was tested whether adding copeptin concentration to the full adjusted model (including age, sex, kidney risk factors, and diuretic use) resulted in significantly better prediction of change in mGFR and change in total kidney volume during follow-up, using linear regression models and the improvement of fit (R^2) of these models (F test).

A 2-sided P<0.05 was considered to indicate statistical significance for all analyses.

RESULTS

A total of 241 patients with ADPKD was enrolled in the CRISP I Study, and from this study, 203 patients re-enrolled in CRISP II. Plasma samples from 225 patients were available for copeptin measurement. Of these patients, 86 were men and 139 were women. Baseline characteristics of these patients are listed in Table 1 by sex-stratified tertiles of plasma copeptin. In general, participating patients were at a relatively early phase of their disease, given a median young age of 33.9 (IQR, 25.1-39.7) years and a (near)-normal median mGFR of 93.9 (IQR, 78.7-144.9) mL/min/1.73 m^2. Median copeptin level was 2.87 (IQR, 1.80-5.10) pmol/L. There was a significant difference in copeptin concentrations between women and men, with men having higher median copeptin values (3.64 [IQR, 2.23-6.40] and 2.43 [IQR, 1.63-4.42] pmol/L, respectively; P = 0.001). Patients with the highest copeptin levels had significant higher total kidney volume and urine osmolality and lower mGFR.

Table 2 lists results of baseline cross-sectional analyses of the associations of plasma copeptin levels with physiologic parameters and measures of ADPKD disease severity. Baseline plasma copeptin concentration was not associated with baseline plasma osmolality or plasma sodium level (R = 0.051; P = 0.5), but was associated with urine osmolality in spot samples obtained at the time of blood collections and baseline blood pressure, mGFR, and total kidney volume. Of note, at baseline, mGFR and total kidney volume correlated well with each other (R = -0.383; P <0.001).

Median follow-up was 8.5 (IQR, 7.7-9.0) years, during which the median change in mGFR was -2.8 (IQR, -5.31 to -0.51) mL/min/1.73 m^2 per year and median percentage of increase in total kidney volume was 5.4% (IQR, 2.8%-9.1%) per year. Mean mGFR and total kidney volume at all times are shown in Figs 1 and 2. Three patients visited the clinic only at baseline. These patients were excluded from longitudinal analyses. Associations between baseline copeptin levels and changes in kidney outcome measures during follow-up are presented in Figs 1 and 2, showing data per sex-stratified tertiles of copeptin concentration (n = 29 men per tertile and n = 46 women per tertile) because median copeptin values were different between men and women. These figures show that the

Figure 1. Mean measured glomerular filtration rate (GFR) at all times in the 3 sex-stratified strata of (A) copeptin level and (B) mean percentage change in measured GFR from baseline. Numbers of patients per time: baseline, n=222; after 1 year, n= 213; after 2 years, n = 208; after 3 years, n = 211; after 6 years, n = 188; and after 8 years, n = 163. Error bars represent 95% confidence intervals.

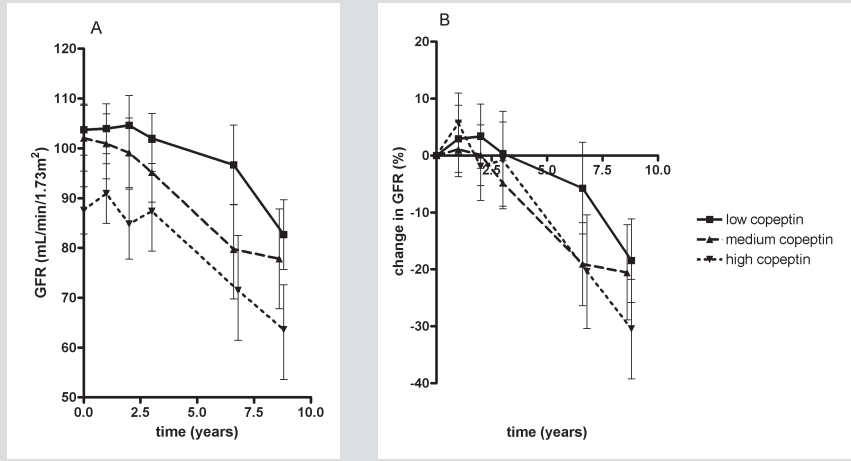
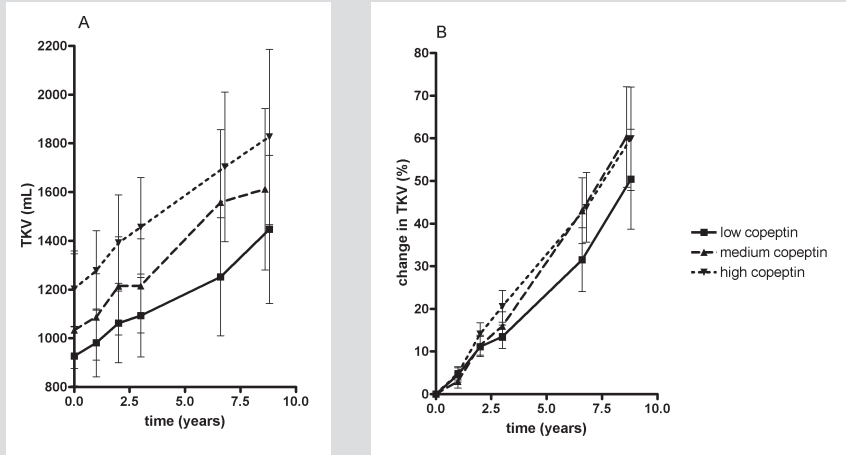


Figure 2. Mean total kidney volume (TKV) at all times in the 3 sex-stratified strata of (A) copeptin level and (B) mean percentage change in measured TKV from baseline. Numbers of patients per time: baseline, n = 225; after 1 year, n = 216; after 2 years, n = 204; after 3 years, n = 214; after 6 years, n = 181; and after 8 years, n = 152. Error bars represent 95% confidence intervals.



higher the baseline copeptin concentration, the more mGFR decreased and the more total kidney volume increased during follow-up.

The association between baseline copeptin concentrations and kidney measures was tested for statistical significance in multivariable regression models. The association between baseline copeptin concentration and change in mGFR was significant after adjusting for sex and age (model 1; P = 0.03), and lost significance after additional adjustment for kidney risk factors (P = 0.09; Table 3). When we adjusted additionally for baseline mGFR, the association was significant (P = 0.004).

Copeptin level was associated significantly with change in total kidney volume during follow-up (P <0.001), also after adjustment for age and sex (P =0.004). This association remained significant after adjusting for kidney risk factors (model 2; P = 0.03; Table 4). When we adjusted additionally for baseline total kidney volume, copeptin level was associated borderline significantly with change in total kidney volume (P = 0.1). We tested a third model in which we adjusted additionally for use of diuretics because this medication influences plasma osmolality and blood volume and, indirectly, AVP. After this adjustment, the association remained the same for change in mGFR (P = 0.09) and change in total kidney volume (P =0.03). When we adjusted in the full multivariable model the association between baseline copeptin level and change in GFR during follow-up not only for baseline mGFR, but also for baseline total kidney volume, the association remained significant (P = 0.007), whereas for change in total kidney volume, the association lost significance (P = 0.4).

When copeptin level was added to the fully adjusted model without copeptin, the adjusted R^2 of the overall model increased from 0.044 to 0.053 for change in mGFR and from 0.200 to 0.214 for change in total kidney volume. Copeptin level was associated more strongly with both change in mGFR and total kidney volume than urine osmolality in all models. Urine osmolality was associated with only change in total kidney volume in the crude model without copeptin level (P = 0.04), but not with change in mGFR or total kidney volume in any of the adjusted models. When urine osmolality was added to the models in addition to copeptin level, it showed that urine osmolality was not associated significantly with change in mGFR or total kidney volume in any of the models.

No interactions were found in the crude or fully adjusted multivariable model between age and copeptin level or between sex and copeptin level in their association with both kidney measures.

When mixed-models analyses were used as sensitivity analysis instead of linear regression analyses, similar results again were obtained for both change in mGFR and change in total kidney volume. In model 2 (adjusted for age, sex, baseline mGFR, and kidney risk factors), the associations with baseline copeptin level yielded β = -0.513 (P = 0.08) for change in mGFR and β = 0.005 (P = 0.008) for change in total kidney volume.

Table 3. Linear regression analysis of the association between baseline copeptin and change in mGFR

	Crude		Model 1		Model 2		Model 3	
	β	p	β	p	β	p	β	p
Ln (copeptin)	-0.626	0.1	-0.884	0.03	-0.716	0.09	-0.723	0.09
Male sex			1.371	0.02	1.623	0.02	1.604	0.02
Ln (age)			-2.726	0.004	-1.916	0.08	-1.982	0.08
Ln (BMI)					-1.305	0.5	-1.372	0.4
MAP (per 1-mm Hg greater)					-0.044	0.1	-0.045	0.1
Ln(HDL-C)					0.218	0.9	0.153	0.9
Smoking (yes)					0.670	0.4	0.620	0.4
Diuretic use (yes)							0.497	0.6

Note: Analyses were adjusted for sex and age (model 1), and additionally adjusted for covariates known to be associated with kidney outcome in autosomal dominant polycystic kidney disease (model 2) and use of diuretics (model 3). Logarithmic transformation of all variables without normal distribution was applied to fulfill the requirement of equal distribution of the residuals. For natural logarithm–transformed measures, copeptin had been measured in pmol/L, age in years, BMI in kg/m², and HDL-C in mg/dL.
Abbreviations: β, standardized beta; BMI, body mass index; MAP, mean arterial pressure; mGFR, measured glomerular filtration rate (adjusted for body surface area); HDL-C, high-density lipoprotein cholesterol.

Table 4. Linear regression analysis investigating the association between baseline copeptin and change in Total Kidney Volume

	Crude		Model 1		Model 2		Model 3	
	β	p	β	p	β	p	β	p
Ln (copeptin)	0.011	<0.001	0.009	0.004	0.006	0.03	0.006	0.03
Male sex			0.017	<0.001	0.010	0.04	0.011	0.02
Ln (age)			-0.012	0.073	-0.016	0.03	-0.021	0.005
Ln (BMI)					0.017	0.2	0.013	0.3
MAP (per 1 mmHg greater)					0.000	0.5	0.000	0.4
Ln (HDL-C)					-0.028	0.003	-0.028	0.002
Smoking (yes)					-0.003	0.6	-0.002	0.8
Diuretic use (yes)							0.020	0.002

Note: Analyses were adjusted for sex and age (model 1) and additionally adjusted for covariates known to be associated with kidney outcome in autosomal dominant polycystic kidney disease (model 2) and use of diuretics (model 3). Logarithmic transformation of all variables without normal distribution was applied to fulfill the requirement of equal distribution of the residuals. For natural logarithm–transformed measures, copeptin had been measured in pmol/L, age in years, BMI in kg/m², and HDL-C in mg/dL.
Abbreviations: β, standardized beta; BMI, body mass index; MAP, mean arterial pressure; HDL-C, high-density lipoprotein cholesterol.

DISCUSSION

These data show that in relatively early-stage ADPKD, copeptin levels, as a marker for AVP, are cross-sectionally not associated with plasma osmolality (which is the most important physiologic stimulus for AVP release), but associate with mGFR and total kidney volume. Furthermore, we found an association between baseline copeptin level and rate of disease progression during follow-up, measured as change in total kidney volume and mGFR over time, independent of age, sex, and kidney risk factors. This association was significant for change in total kidney volume and borderline significant for change in mGFR.

We previously showed in a cohort of 102 patients with ADPKD that copeptin level was associated cross-sectionally with markers of disease severity, and in another cohort of 79 patients with ADPKD, that higher copeptin levels were associated with a decrease in GFR during follow-up [9]. The present study differs in 3 aspects from these previous studies. First, the present study has information about important covariates that allow study of the association between copeptin level and physiologic variables and allow adjustment in multivariable regression analyses. Second, patients in a relatively early phase of disease were included. Third, not only GFR, but also total kidney volume was measured to assess disease progression. Therefore, this study corroborates previous findings and adds important new information.

At baseline, we found no association between plasma osmolality and copeptin level in this study. Interestingly, in the 2 studies that were performed in patients without ADPKD that investigated the association between plasma osmolality and copeptin level, fundamentally different results were obtained. One study was performed in a general population cohort [19], and the other, in kidney transplant recipients [20]. Both studies showed that in accordance with normal physiology, the higher the plasma osmolality, the higher the copeptin level [19,20]. These data taken together suggest that in ADPKD, copeptin (and thus AVP) concentration is not under normal control of its physiologic stimulus (plasma osmolality), although it still exerts its physiologic effects (urine osmolality). However, in an earlier study of patients with ADPKD, we found a significant association between copeptin level and plasma osmolality [8]. This study was performed in a different population (broader GFR range). Given this previous observation, it is difficult to conclude whether the relation between plasma osmolality and copeptin level is disturbed in ADPKD. Interestingly, we found at baseline an association between total kidney volume, a measure of ADPKD severity, and copeptin level.

In the previous study in which we showed that in patients with ADPKD, copeptin level is associated with rate of kidney function decrease, individuals were included with a wide estimated GFR range (22-120 mL/min/1.73 m^2) [9]. It could be objected that in that study, higher copeptin levels are the result of decreased renal clearance, bringing the question forward of what happens first: is it copeptin level that increases to predict a decrease in GFR or is it decreased GFR leading to higher copeptin values? Unfortunately, there is no literature about how copeptin is cleared from the body. Copeptin has a molecular weight

of 5 kDa [5] and consequently is subject to glomerular filtration. Decreased renal clearance therefore theoretically may lead to higher copeptin values, which might influence our results. However, it is known from other studies that copeptin values can decrease very quickly after a water load [5], suggesting extrarenal clearance as the predominant clearance mechanism. Another fact that favors that first copeptin level increases and then GFR decreases is that in the present study, participants were included who were at a relatively early stage of their disease, indicated by the young average age and near-normal mGFR at baseline (median, 93.9 mL/min/1.73 m^2). Even in this population, higher copeptin levels were found to be predictive for kidney measures. These data are in line with another recent finding. A water deprivation test was performed in 15 healthy controls and 15 individuals with ADPKD with normal kidney function. Estimated GFR in both groups was similar (104 vs 100 mL/min/1.73 m^2; 24-hour creatinine clearance, 117 vs 116 mL/min). It was shown that these patients with ADPKD already had decreased urinary concentrating capacity, and plasma osmolality was maintained within the normal range at the cost of higher copeptin and AVP levels [21]. In combination, this latter study and the present study suggest that copeptin (and thus AVP) levels increase before GFR decreases and therefore is an early marker.

Because patients with ADPKD in the present study were included in a relatively early disease stage, only limited GFR loss was to be expected (despite the 8.5 years of follow-up). The rate of GFR loss was limited (median value, -2.8 mL/min/1.73 m^2 per year). It is widely accepted that in patients with ADPKD, GFR remains fairly stable during the first decades of life because of compensatory hyperfiltration, whereas in this period, total kidney volume steadily increases [22,23], as also suggested by Fig 2. In our study, we found that the association between baseline copeptin level and mGFR decrease reached significance only after adjustment for sex and age (model 1, Table 3). It is known that men and older patients with ADPKD have more GFR decrease than women and younger patients. This may explain why the association between copeptin level and change in mGFR become significant only after adjustment for these factors. In multivariate models 2 and 3, the association between copeptin level and change in mGFR was of limited strength and reached statistical significance only after additional adjustment for baseline mGFR. In contrast, results with respect to total kidney volume were more robust. These data therefore are in line with the assumption that total kidney volume is a valuable surrogate measure to assess (progression of) disease severity, especially in early ADPKD [14].

Our findings may have 2 consequences. First, they illuminate the pathophysiologic mechanism, which helps clarify disease progression in ADPKD. Second, these data suggest that copeptin may be a valuable marker to predict disease progression in clinical practice. Previously, disease progression in ADPKD has been hypothesized [8] to begin with anatomical disruption of medullary architecture due to cyst formation arising from a genetic mutation and consequently to an impairment in medullary urea gradient, which causes decreased urinary concentrating capacity. In order to maintain fluid balance and

keep plasma osmolality within normal ranges, AVP level increases. AVP, when bound to the V2 receptor at collecting duct cells, causes an increase in intracellular cAMP, which then leads to proliferation of these epithelial cells and chloride-driven fluid secretion into cysts. As a result, a vicious circle arises that predisposes for cyst formation, cyst growth, and kidney function loss. Although our present findings show that relatively early in the disease course, copeptin (as surrogate for AVP) is not associated with plasma osmolality, they support the hypothesis that copeptin is associated with total kidney volume and predicts the rate of growth of total kidney volume and rate of GFR loss. The alleged pathophysiologic role of AVP is strengthened further by findings in experimental models [24–28], as well as by findings in a post hoc analysis of 2 open-label clinical studies of patients with ADPKD [29]. Both suggest that treatment with a V2 receptor antagonist ameliorates disease progression in ADPKD. At present, a large-scale, prospective, double-blind, randomized clinical trial is investigating the efficacy of a V2 receptor antagonist to halt disease progression in ADPKD [30]. This trial is expected to provide the definitive answer to the question of whether AVP is causally related to the rate of disease progression in these patients.

From this study, it cannot be concluded that copeptin is a marker for disease progression in ADPKD specifically or that it is a marker for chronic kidney disease progression in general. We recently measured copeptin in cohorts of kidney transplant patients [20] and patients with diabetes (Boertien et al, unpublished data, 2012). Also in these patients, a higher copeptin level at baseline was found to be associated with worse kidney measures during follow-up. Therefore, we hypothesize that high copeptin levels also may have pathophysiologic significance in non-ADPKD kidney disease.

Our data furthermore indicate that copeptin may be a valuable novel biomarker to identify patients with ADPKD at risk of accelerated disease progression, even relatively early in the course of the disease. Of note, there is large variability in the rate of disease progression between individuals, even within families that share the same mutation causing ADPKD. The drugs that at present are under investigation as possible renoprotective agents in ADPKD (V2 receptor antagonists and somatostatin analogues) have a considerable side-effect profile [31,32] and have been suggested to be especially effective in the earlier phase of ADPKD [24]. Distinguishing in this early phase between individuals at lower risk from those at higher risk of accelerated disease progression may help select patients in whom it is reasonable to expose them to drugs that are associated with side effects. However, it should be noted that there is considerable overlap in the rate of increase in total kidney volume and decrease in mGFR between groups with high versus low copeptin levels (Figs 1 and 2). Copeptin level as prognostic marker therefore probably is useful only in case of a low or high value or in combination with other early prognostic markers, such as total kidney volume and genotype. Assessing the exact predictive value of copeptin, alone or in concert with these other prognostic markers, is beyond the scope of the present study and should be the aim of studies specifically dedicated to this research question.

We acknowledge that this study has limitations. First, because of missing plasma samples, copeptin was not measured in some patients (n = 16). This is not expected to lead to bias because samples were missing due to a random process. Second, blood samples were drawn under protocolized circumstances, but patients were allowed to drink ad libitum. Differences in hydration status between individuals will lead to variability in copeptin concentration (as a marker of AVP). However, this is expected to lead to effect dilution and therefore to under- rather than overestimation of the association between copeptin level and rate of disease progression. Furthermore, the measured copeptin value is more likely to be representative during such a normal hydration status.

Strengths of this study are the relatively large number of patients with ADPKD who are well phenotyped and the availability of measures to judge disease progression during follow-up that currently are accepted as the golden standards, being GFR assessed as iothalamate clearance and total kidney volume assessed by MRI and the long-term median follow-up of 8.5 years.

From this study it can be concluded that in patients with ADPKD, relatively early in their disease, copeptin as marker for AVP is not associated with plasma osmolality but with markers of disease severity. Importantly, high baseline copeptin levels are associated independently with an increase in total kidney volume and decrease in GFR during follow-up. Copeptin therefore might be an early easy-to-measure marker that may help predict outcome in ADPKD. These data furthermore are in line with the alleged pathophysiologic role of vasopressin in ADPKD.

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PART 2

6

VASOPRESSIN, COPEPTIN, AND RENAL CONCENTRATING CAPACITY IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE WITHOUT RENAL IMPAIRMENT

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ABSTRACT

Background and objectives. Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent hereditary renal disease, characterized by cyst formation in the kidneys leading to end stage kidney failure. It is clinically acknowledged that ADPKD patients have impaired urine concentrating capacity, but the mechanism behind this observation is unknown.

Design, setting, participants, & measurements. Fifteen ADPKD patients (estimated GFR ≥60 ml/min per 1.73m^2) and 15 age- and sex-matched healthy controls underwent a standard prolonged water deprivation test in which urine and plasma osmolality, vasopressin, and copeptin were measured. The effect of a synthetic vasopressin analog (desmopressin) injected at the moment of maximal urine concentrating capacity was also studied.

Results. After 14 hours of water deprivation, ADPKD patients tended to have higher plasma osmolality (P=0.07) and significantly higher vasopressin and copeptin levels (both P<0.05), whereas urine osmolality was similar in ADPKD patients and controls (710 versus 742 mOsmol/kg; P=0.61). Maximal urine concentrating capacity was lower in ADPKD patients (758 versus 915 mOsmol/kg in controls; P<0.001). At maximal urine concentrating capacity, plasma osmolality, vasopressin, and copeptin levels were significantly higher in ADPKD patients. The median increase in urine osmolality after desmopressin administration in ADPKD patients was less than in healthy controls.

Conclusions. Already early in their disease, ADPKD patients have impaired maximal urine concentrating capacity brought out upon dehydration, with no evidence of impaired hypothalamic response. To maintain fluid balance, vasopressin concentration increases, which is hypothesized to play a role in ADPKD disease progression.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease, with an estimated prevalence of approximately 1 in 1000. ADPKD is characterized by progressive bilateral cyst formation in the kidneys, leading to pain, hematuria, and end stage kidney failure that usually occurs in the fourth to sixth decade of life [1].

The pathogenetic mechanisms responsible for cyst formation in ADPKD are complex [1]. Due to a genetic defect in the polycystin complex of the primary cilium, intracellular calcium concentration is reduced in cells of the collecting tube, which results in increased levels of intracellular cAMP [2-4]. cAMP is an important player in cyst formation, causing proliferation of tubular cells and chloride-driven fluid secretion into cysts [5].

Arginine vasopressin (AVP) is assumed to have a detrimental role in the pathogenesis of ADPKD. Production of cAMP by adenylyl cyclase is enhanced when AVP is bound to the vasopressin V2 receptor at the basolateral side of collecting tube cells, causing cyst enlargement via the aforementioned mechanisms [6]. In line with these assumptions are experimental studies showing that a vasopressin V2 receptor antagonist decreases the rate of cyst formation [7-9]. Clinical trials are ongoing to examine the effect of vasopressin V2 receptor antagonists in ADPKD patients [10].

Despite this alleged pivotal role of AVP in ADPKD, surprisingly little is known about AVP levels in ADPKD patients. It is, however, clinically well acknowledged that ADPKD patients cannot concentrate their urine well [11]. This effect can be observed at a young age [12-14]. The mechanism behind this decreased urine concentrating capacity is not known, but it is suggested to have a renal origin. The impaired ability to reabsorb water could be secondary to cyst-induced abnormality in renal architecture, leading to an impaired medullary osmotic gradient [15] or to insensitivity to AVP (e.g., due to a receptor defect) [4,16]. Theoretically, a lower renal concentrating capacity could also have a central cause (i.e., impaired AVP release by the pituitary gland).

Given this background, we hypothesized that ADPKD patients have an impaired renal concentrating capacity, leading to an increase in plasma AVP levels as a compensatory response. To test this hypothesis, we performed a water deprivation test in ADPKD patients early in their disease, and in age- and sex-matched healthy controls, in which we measured urine and plasma osmolality as well as plasma concentrations of AVP and copeptin (part of the precursor hormone of AVP). In addition, we studied the effect of an injection of a synthetic AVP analog, desmopressin (DDAVP), at the moment of maximal urine concentrating capacity to determine whether an impaired hypothalamic response is involved.

MATERIALS AND METHODS

Study Population

ADPKD patients with a diagnosis based on the criteria of Ravine et al. [17] and healthy controls aged between 18 and 65 years were eligible for this study. An additional inclusion criterion for both groups was an estimated GFR (eGFR) ≥60 ml/min per 1.73 m² to exclude a renal urine concentrating defect that can be observed in participants with a low GFR [18]. Exclusion criteria were as follows: use of medication that influences renal concentration capacity, such as diuretics and postmenopausal hormone therapy; history of diseases influencing renal concentration capacity, such as diabetes mellitus, diabetes insipidus, adrenal or thyroid deficiencies, or kidney diseases other than ADPKD; other factors that can influence renal concentration capacity such as smoking, menstruation, urinary tract infection, pregnancy, and consumption of ≥4 alcohol beverages per day; and active cardiovascular disease, which is a contraindication for DDAVP administration.

Healthy controls were matched for age (within 5 years) and sex with ADPKD patients. A healthy individual was defined according the aforementioned criteria and had no evidence of CKD (eGFR ≥60 ml/min per 1.73 m², albuminuria <30 mg/d, and no plasma electrolyte abnormalities).

This study was approved by our institutional review board and was performed in adherence to the Declaration of Helsinki. All participants gave written informed consent.

Study Protocol

Before the water deprivation test, urine was collected for 24 hours and blood was drawn for measurement of albuminuria, creatinine clearance, AVP, and copeptin. Eligible ADPKD patients and healthy controls underwent a standard prolonged water deprivation test, based on the protocol originally described by Miller et al. [19]. The day before the water deprivation test, participants were not allowed to smoke or consume caffeine-containing products. Participants received a standard meal and were not allowed to eat or drink after 6 p.m. During an in-hospital visit the next day, urine specimens were collected every hour and blood samples were taken every 2 hours from 8 a.m. onward until urine osmolality became constant, defined as an increase in urine osmolality between two consecutive urine collections <30 mOsml/kg. After reaching this plateau, participants received an intramuscular injection of 2 µg of DDAVP. Two hours after injection, blood and urine samples were again collected. Thereafter, participants were allowed to drink and eat ad libitum. The stopping criteria during the water deprivation test to ensure patient safety were as follows: reaching a body weight reduction >3% compared with body weight measured at 6 p.m. the day before, or a plasma sodium >150 mmol/L any time during the study.

Interpretation of a Water Deprivation Test

According to the standard criteria, a water deprivation test [19,20] is considered normal when urine osmolality is >800 mOsm/kg at plateau. Complete central nephrogenic diabetes insipidus can be expected in patients with urine osmolality <300 mOsm/kg at plateau and a >50% increase in urine osmolality after DDAVP administration. Partial central diabetes insipidus is expected in participants with a maximum urine osmolality between 300 and 800 mOsm/kg and a 9%–50% increase in urine osmolality after DDAVP administration. Complete renal diabetes insipidus is expected in participants with urine osmolality <300 mOsm/kg at plateau and a <9% increase in urine osmolality after DDAVP administration, whereas partial renal diabetes insipidus is suspected in participants with a maximum urine osmolality between 300 and 800 mOsm/kg and a <9% increase in urine osmolality after DDAVP administration.

MEASUREMENTS

Standard biochemical evaluation was performed in fresh urine and plasma samples, using a Roche Modular Autoanalyser (Hitachi, Tokyo, Japan). GFR was estimated with the Chronic Kidney Disease Epidemiology Collaboration equation [21]. Plasma and urine osmolality were measured directly via determination of freezing point depression using an Osmometer (Arkray, Kyoto, Japan), with a variation coefficient <1.0%.

Blood for plasma AVP measurement was taken into a chilled syringe, placed in a chilled lithium heparin container, and immediately centrifuged at 4°C and stored at -80°C until assay. AVP was measured by RIA after an extraction using ODS-silica (DiaSorin, Stillwater, MN) in the General Clinical Laboratory of the IJsselland Hospital (Capelle aan de IJssel, The Netherlands). The assay range was between 0.2 and 4.7 pg/ml, with a sensitivity of 0.2 pg/ml with 2.5 ml of plasma. The average duplo coefficients of variation were 4.3% for the low range (0.2–0.4 pg/ml), 4.7% for the intermediate range (0.4–1.0 pg/ml), and 3.5% for the high range (1.0–8.1 pg/ml), respectively.

Plasma samples for copeptin measurement were taken into EDTA tubes, and the peptide was measured using a sandwich immunoassay (B.R.A.H.M.S. AG, Hennigsdorf/ Berlin, Germany). The lower limit of detection was 0.4 pmol/L and the functional assay sensitivity (interassay coefficient of variation <20%) was <1 pmol.

STATISTICAL ANALYSES

A power analysis was performed to determine how many participants were to be included in this study. The literature provides no data on AVP levels in ADPKD patients at an early stage of their disease, the primary parameter of interest. We therefore powered this

study based on maximal urine concentrating capacity of 812 ±144 mOsmol/kg in healthy individuals and 680 ±186 mOsmol/kg in ADPKD patients [15]. These data, adopting a 5% two-sided a and 80% power, indicated that at least 15 healthy participants and 15 ADPKD patients were needed to show a significant difference in urine concentrating capacity between ADPKD patients and healthy participants.

Parametric variables are expressed as mean ± SD, whereas nonparametric variables are given as median (interquartile range). P values for differences between ADPKD patients and healthy controls were tested using a chi-squared test for categorical data as well as a t test for parametrical and a Mann–Whitney U test for nonparametric continuous data. To test correlations between AVP and copeptin, both variables were log-normalized and Pearson's regression analysis was used. All analyses were performed using the SPSS statistical package (version 18.0; SPSS Inc, Chicago, IL). A two-sided P value <0.05 was considered statistically significant.

RESULTS

Characteristics of the participating patients and healthy controls are presented in Table 1. Fifteen ADPKD patients and 15 age- and sex-matched healthy controls were studied.

Importantly, ADPKD patients and healthy controls had similar kidney function, but albuminuria was higher in ADPKD patients. ADPKD patients had similar BP compared with healthy controls, but used antihypertensive medications more often. Per protocol, none of the participating participants used diuretics. Lastly, AVP and copeptin levels were higher in ADPKD patients compared with healthy controls but not significantly.

Table 2 shows the results of the prolonged water deprivation test. All subjects completed the water deprivation test without any complications (i.e., none of the stopping criteria were met).

At 8:00 a.m. (after 14 hours of water deprivation), ADPKD patients tended to have higher plasma osmolality (P=0.07) and significantly higher plasma AVP and copeptin levels compared with healthy controls (P=0.03 and P=0.04, respectively), whereas urine osmolality was not different between the two study groups at this time point (P=0.61). The higher plasma osmolality at 8:00 a.m. in ADPKD patients was primarily due to higher plasma urea levels (P=0.002), with plasma sodium levels being comparable between both study groups (P=1.00).

Maximal urine concentrating capacity was reached after a median of 16 hours in ADPKD patients and 17 hours in healthy controls (P=0.02). Maximal urine concentrating capacity was significantly lower in ADPKD patients compared with healthy controls (P<0.001) (Table 2, Figure 1). The difference in maximal urine osmolality between ADPKD patients and healthy controls was related to a difference in urine urea concentration, whereas urine sodium concentration was not different between both study groups (P=0.25). Nine

Table 1: Characteristics of ADPKD patients and age- and gender matched healthy controls.

	ADPKD N=15	Healthy controls N=15	p-value
Age (y)	36±15	35±12	0.93
Male (%)	47	47	1.00
BMI (kg/m²)	27±5	25±4	0.33
MAP (mmHg)	100±7	96±21	0.59
Using antihypertensives (%)	53	0	0.001
eGFR (mL/min/1,73 m²)	100±23	104±12	0.62
Creatinine clearance (mL/min/1.73m²)	116±35	117±19	0.97
Urine volume (L/24h)	2.00±0.65	2.20±0.99	0.63
Urine osmolality (mOsmol/kg/24h)	548±149	495±209	0.45
Urine albumin (mg/24h)	30.0 (16.0-124.0)	3.0 (2.0-5.0)	<0.001
Plasma AVP (pgl/mL)	1.34 (0.25-3.07)	0.87 (0.28-2.38)	0.19
Plasma copeptin (pmol/L)	8.92 (0.66-21.86)	6.08 (0.92-10.79)	0.22

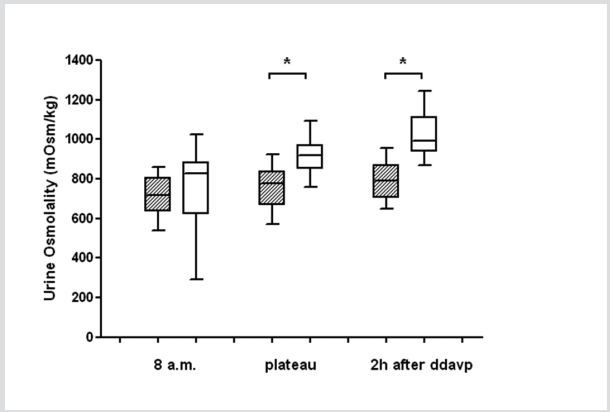
Data are given as means ± SD for parametric data or medians (IQR) for non-parametric data. Significance was tested using a chi-square test, Student's t-test or a Mann-Whitney U test, when appropriate. Abbreviations are: BMI, body mass index; eGFR, estimated glomerular filtration rate; MAP, mean arterial pressure.

Table 2: Characteristics of ADPKD patients and age and sex matched healthy controls at 8:00 a.m. (after 14 hours of water deprivation), when reaching plateau (increase in urinary osmolality between two consecutive urine collections less than 30 mOsmL/kg), and after DDAVP administration during a standard prolonged water deprivation test.

	ADPKD N=15	Healthy controls N=15	p-value
8:00 a.m. (14 hr water deprivation)			
Plasma osmolality (mOsmol/kg)	285±5	282±3	0.07
Plasma sodium (mmol/L)	141.2±1.6	141.2±1.0	1.00
Plasma urea (mmol/L)	6.5±1.9	4.7±1.0	0.002
Urine osmolality (mOsmol/kg)	710±103	742±216	0.61
Urine sodium (mmol/L)	111.4±41.0	103.9±41.7	0.75
Urine urea (mmol/L)	333±90	366±143	0.46
Plasma AVP (pgl/mL)	1.08 (0.66-3.75)	0.43 (0.40-0.81)	0.03
Plasma copeptin (pmol/L)	14.85 (4.92-18.68)	8.48 (2.90-9.37)	0.04
Plateau (maximal urinary concentrating capacity)			
Plasma osmolality (mOsmol/kg)	285±4	282±3	0.02
Plasma sodium (mmol/L)	141.5±1.8	141.0±1.5	0.39
Plasma urea (mmol/L)	6.5±1.9	4.6±0.9	0.002
Urine osmolality (mOsmol/kg)	758±103	915±91	<0.001
Urine sodium (mmol/L)	138.7±46.7	119.4±36.5	0.25
Urine urea (mmol/L)	312±87	400±102	0.02
Plasma AVP (pgl/mL)	1.26 (0.94-2.58)	0.47 (0.36-1.06)	0.004
Plasma copeptin (pmol/L)	14.74 (7.47-18.96)	4.62 (3.42-7.84)	0.01
2 hr after DDAVP administration			
Plasma osmolality (mOsmol/kg)	285±4	282±3	0.03
Plasma sodium (mmol/L)	141.5±1.6	141.9±1.9	0.54
Plasma urea (mmol/L)	6.6±1.8	4.8±0.9	0.002
Urine osmolality (mOsmol/kg)	790±99	1015±114	<0.001
Urine sodium (mmol/L)	141.1±47.5	120.1±45.1	0.76
Urine urea (mmol/L)	280±56	405±110	0.001
Plasma AVP (pg/mL)	1.7 (1.13-2.41)	0.92 (0.72-2.15)	0.07
Plasma copeptin (pmol/L)	17.01 (7.94-17.78)	7.75 (3.81-8.80)	0.04

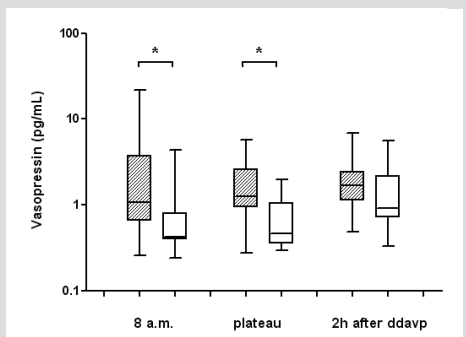
Data are given as means ± SD for parametric data or as medians (IQR) for non-parametric data. Significance was tested using Student's t-test or a Mann-Whitney U test, when appropriate. Abbreviations are: AVP, arginine vasopressin; DDAVP, desmopressin.

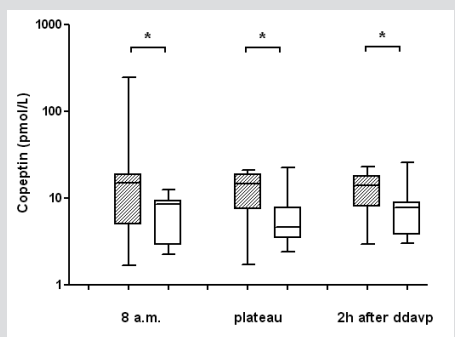
Figure 1: Urine osmolality in ADPKD patients (N=15, hatched boxes) and healthy controls (N=15, open boxes) during a standard prolonged water deprivation test. Data are given as box plots, showing median values, interquartile ranges and ranges of distribution, at 8 a.m. in the morning (i.e. after 14hr of water deprivation), at plateau (i.e. when stable urine osmolality was reached), and two hours after DDAVP administration. *, p<0.05.



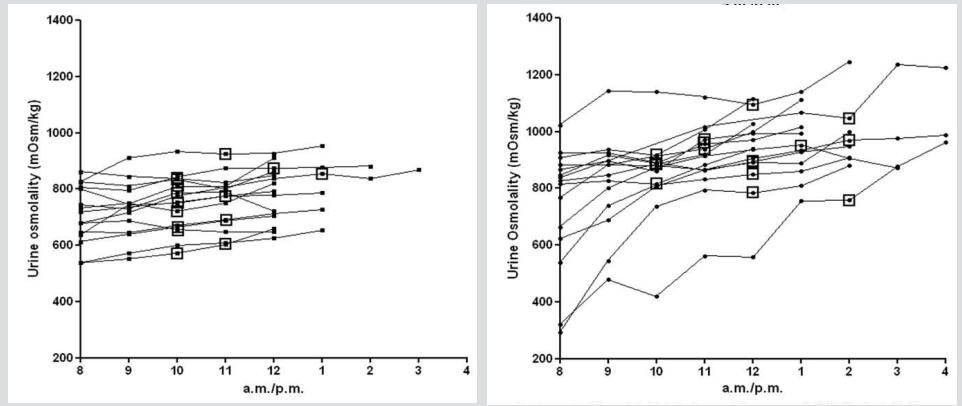
of 15 ADPKD patients did not reach a urine osmolality >800 mOsmol/kg, whereas this was the case in only 2 of 15 healthy controls (60% versus 13%; P<0.03). A significant difference was found in maximal urinary concentrating capacity between ADPKD patients taking antihypertensive drugs (697±96 mOsmol/kg) and normotensive ADPKD patients (826±60 mOsmol/kg; P=0.009). Maximal urinary concentrating capacity was significantly lower in normotensive ADPKD patients (826±60 mOsmol/kg) compared with healthy controls (915±91 mOsmol/kg; difference P=0.03). At the time point of maximal urine concentrating capacity, ADPKD patients had significantly higher plasma osmolality as well as higher plasma AVP and copeptin levels (P=0.02, P=0.004, and P=0.01, respectively) (Figure 2).

Figure 2: Plasma vasopressin (upper panel) and copeptin (lower panel) in ADPKD patients (N=15, hatched boxes) and age and healthy controls (N=15, open boxes) during a standard prolonged water deprivation test. Data are given as box plots, showing median values, interquartile ranges and ranges of distribution, at 8 a.m. in the morning (i.e. after 14hr of water deprivation), at plateau (i.e. when stable urine osmolality was reached, and two hours after DDAVP administration). *, p<0.05.





Supplementary Figure 1: Individual urine osmolality responses in ADPKD patients (N=15, upper panel) and healthy controls (N=15, lower panel) during a standard prolonged water deprivation test. Open squares represent the time point at which DDAVP was administered.

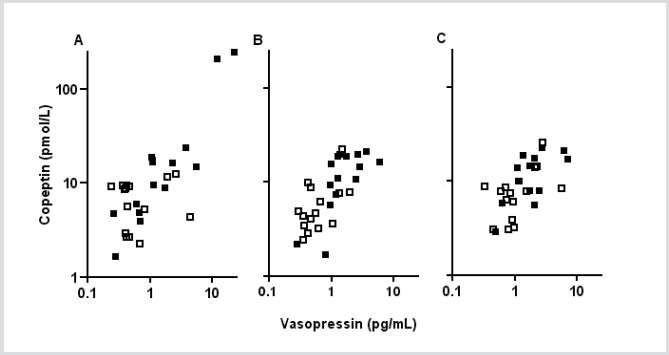


After DDAVP injection, median urine osmolality increased slightly in ADPKD patients (3.2%; interquartile range, 1.2%–7.7%; P=0.009) as well as healthy controls (12.0%; interquartile range, 5.8%–17%; P=0.002), but significantly less in ADPKD patients (difference P<0.02). Only two of the nine ADPKD patients that had a maximal urine concentrating capacity <800 mOsmol/kg showed a >9% increase in urine osmolality after DDAVP injection, whereas such a rise was observed in both healthy controls that had not reached a urine osmolality >800 mOsmol/kg on thirsting. Plasma osmolality and copeptin concentration were significantly higher in ADPKD patients after DDAVP injection compared with healthy controls (P=0.03 and P=0.04, respectively) and AVP tended to be higher in ADPKD (P=0.07). Individual responses to the water deprivation test are shown in Supplemental Figure 1.

Measurements of AVP and copeptin yielded similar results during the entire water deprivation test (Table 2).Moreover, significant associations were found between plasma AVP and copeptin levels at 8:00 a.m. (R^2=0.58, P<0.001), plateau (R^2=0.58, P<0.001), and 2 hours after DDAVP administration (R^2=0.40, P<0.001). Plots showing the associations between AVP and copeptin are given in Figure 3.

Total kidney volume (TKV) of 10 patients based on magnetic resonance imaging was available, with a median of 936 ml (interquartile range, 849–1614). Associations (R^2) of TKV with AVP and copeptin in these 10 patients were 0.47 (P=0.03) and 0.22 (P=0.17), respectively.

Figure 3: Strong associations between plasma vasopressin and plasma copeptin during water deprivation. (A) Association at 8:00 a.m. (after 14 hours water deprivation), (B) at plateau (when stable urine osmolality was reached), and (C) 2 hours after desmopressin administration. Results are shown separately for patients with autosomal dominant polycystic kidney disease (n=15, solid squares) and controls (n=15, open squares).



DISCUSSION

In this study, we performed a standard prolonged water deprivation test in 15 ADPKD patients and 15 age- and sex-matched healthy controls and measured AVP and copeptin levels. Our findings of an impaired concentrating mechanism, brought out upon dehydration, in ADPKD patients are in agreement with the literature [12-15, 22, 23]. It was also emphasized that this impaired concentrating capacity was already present early in the disease course [12-14]. Gabow et al. observed that cyst number and size, and remaining volume of normal parenchyma were associated with greater impairment of urine concentrating capacity [15]; thus, the impaired urine concentrating capacity is thought to be caused by an impaired medullary osmotic gradient due to distorted renal architecture by cyst formation. There are important differences between this study and the previously performed studies. All previous studies measured urine osmolality at a fixed time period after water deprivation (and concurrent DDAVP administration in most of them), whereas we measured urine osmolality in consecutively collected urine samples and studied the effect of DDAVP administration when participants had reached maximal endogenous concentrating capacity. This procedure allows us to study whether a central component is involved when impaired renal concentrating capacity is found. Furthermore, unlike the previous studies, we measured AVP and copeptin concentrations under the standardized circumstances of dehydration to test the consequences of the impaired concentrating mechanism in view of the suggested unfavorable long-term effects of increased AVP concentration.

After 14 hours of water deprivation, plasma osmolality was significantly higher in ADPKD patients compared with healthy controls with similar age, sex distribution, and kidney function. Plasma AVP was also increased at that time point, whereas urine osmolality in these patients was still similar to urine osmolality in healthy controls. AVP seemed to increase to maintain fluid balance. Maximal urine concentrating capacity was impaired in the ADPKD patients, with 9 of the 15 patients not reaching a urine osmolality >800 mOsmol/kg. At the time point of maximal urine concentrating capacity, ADPKD patients again had higher plasma osmolality and higher AVP. DDAVP administration increased urine osmolality slightly in ADPKD patients, with only two of the nine patients that had a maximal urine concentrating capacity <800 mOsmol/kg showing a >9% increase in urine osmolality after DDAVP injection. Importantly, the median increase in urine osmolality after DDAVP administration in ADPKD patients was less than in healthy controls. These data should be interpreted as an impaired renal concentrating capacity with no evidence for a central component (i.e., impaired AVP release by the pituitary gland).

AVP is assumed to have a specific detrimental role in the pathogenesis of ADPKD. Despite this alleged pivotal role of AVP, surprisingly little is known about AVP levels in ADPKD patients. Data have shown AVP levels to be increased in participants with impaired kidney function due to non-ADPKD kidney disease [24]. To our knowledge, only two studies have measured AVP levels in ADPKD patients and both showed increased AVP levels compared with healthy controls [25, 26]. To note, both studies included ADPKD patients with impaired kidney function. We therefore corroborate these findings, but extend the present knowledge on AVP levels in ADPKD by showing that AVP levels are already elevated in the early stages of the disease, because ADPKD patients and healthy controls had similar kidney function in our study. The increase in AVP levels should be interpreted as a compensatory mechanism to maintain fluid balance.

The fact that there are only limited data on AVP in ADPKD may be because AVP is difficult to measure due to its small size, binding to platelets, and very short ex vivo t1/2 [27, 28]. An assay was recently developed to measure copeptin [29]. Copeptin is a part of the precursor of AVP, preprovasopressin, and has been suggested to be a relatively easy to measure, more stable (ex vivo), and reliable marker of AVP secretion [30]. We therefore also measured copeptin in this study. Results with respect to copeptin during the water deprivation test closely mimic the results with respect to AVP. Moreover, significant associations were found between copeptin and AVP concentrations during all three time points on which these variables were measured. Copeptin seems therefore a reliable substitute for AVP in participants with ADPKD. Interestingly, copeptin has recently been investigated in several epidemiologic studies, among others in ADPKD patients [20, 31-33] (W.E. Boertien et al., unpublished observations). In a cross-sectional study in 102 ADPKD patients, we found that copeptin levels were associated with various markers of disease severity, among which albuminuria, GFR, renal blood flow, and total renal volume [33]. In a prospective study in 79 ADPKD patients, we subsequently showed that baseline copeptin

levels were associated with faster kidney function decline when assessed as the change in either iothalamate clearance during short-term follow-up or eGFR during long-term follow- up (W.E. Boertien et al., unpublished observations). From these studies, it was not clear whether copeptin levels are higher in ADPKD patients compared with healthy controls, nor whether a rise in copeptin precedes disease progression or is merely a marker of impaired kidney function. This study, in which copeptin levels were measured under standardized circumstances, provides this information and shows that copeptin levels are already elevated in ADPKD patients with normal kidney function.

Our findings may help shed light on a pathophysiologic mechanism causing disease progression in ADPKD. We previously hypothesized [33] that cysts are formed due to a genetic defect, leading to disturbance of medullary architecture and consequently to impaired urine concentrating capacity early in the disease when kidney function is still normal. As compensatory mechanism AVP levels increase to maintain fluid balance, AVP in turn causes increased levels of cAMP in collecting tube cells [34], leading to proliferation of tubular cells and chloride-driven fluid secretion into cysts [5,35]. Thus, a vicious circle may arise leading to further cyst formation, cyst growth and kidney function decline. This hypothesis is supported by the fact that AVP was found to be increased at normal kidney function in our study and that copeptin, a surrogate for AVP, was shown to predict kidney function decline in another study.

Strengths of this study are that we included ADPKD patients and age- and sex-matched healthy controls with similar kidney function. This allowed us to conclude whether differences between ADPKD patients and healthy controls were due to the disease process itself, and not due to differences in age, sex distribution, or kidney function. These latter factors have been shown to influence maximal urine concentrating capacity [24, 36-39]. Second, we measured maximal endogenous urine concentrating capacity as well as the reaction to DDAVP administration. By administering DDAVP, a central component contributing to decreased maximal urine concentrating capacity could be made unlikely. Third, in addition to urine and plasma osmolality, we also measured AVP and copeptin concentrations. A limitation is that a relatively small number of ADPKD patients and healthy controls were included. However, we performed a power analysis before the start of the study and we included this number of participants. Furthermore, our results showed significant differences for the primary outcome variables. It is therefore unlikely that different conclusions would have been reached if more participants had been included. Fourth, we did not measure per protocol TKV in this study. An association of TKV with AVP and copeptin levels could therefore only be assessed in 10 patients. TKVs used in our association were measured approximately 3 years before this study. However, in a previous study [33], we showed that a higher TKV was independently associated with higher copeptin levels. Last, we observed a significant difference in urinary concentrating capacity between ADPKD patients taking antihypertensive drugs and normotensive ADPKD

patients. An extensive literature search did not provide any evidence that antihypertensive medication (besides diuretics) can influence maximal urinary concentrating capacity. Per protocol, the use of diuretics was not allowed. We interpret this difference therefore as being the result of the fact that ADPKD patients that are hypertensive have more severe disease, and consequently also have more impaired urinary concentrating capacity. To note, we also observed a significant difference in urinary concentrating capacity between normotensive ADPKD patients and healthy controls. This strengthens our findings that patients still early in their disease have an impaired urinary concentrating capacity.

In conclusion, ADPKD patients already have impaired urine concentrating capacity, brought out upon dehydration, in the early stage of their disease. This study shows that a central component in this abnormality is unlikely. Furthermore, we found that AVP and copeptin levels are already elevated after 14 hours of dehydration in this early disease stage, and thus precede kidney function decline in ADPKD patients. In cases in which AVP is indeed causally linked to cyst formation, cyst growth, and kidney function decline, these data provide support for a pathophysiologic concept that may help explain disease progression in ADPKD.

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DISCLOSURES

None.

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SHORT-TERM RENAL HEMODYNAMIC EFFECTS OF TOLVAPTAN IN SUBJECTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE AT VARIOUS STAGES OF CHRONIC KIDNEY DISEASE

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ABSTRACT

Vasopressin V2-receptor antagonists may delay disease progression in ADPKD. Trials with V2-receptor antagonists have been performed predominantly in patients with an estimated creatinine clearance of 60 ml/min or more. Here we determined renal hemodynamic effects of the V2-receptor antagonist tolvaptan in 27 patients with ADPKD at various stages of chronic kidney disease: group A: >60, group B: 30–60, and group C: <30 ml/min per 1.73m^2. Measurements were performed before, after 3 weeks of tolvaptan (up titration to 90/30mg/day, split dose), and 3 weeks after the last dose of tolvaptan. With tolvaptan, a minor, reversible decrease in GFR (^{125}I-iothalamate clearance) was found that reached significance in groups A and B: -7.8 (interquartile range -13.7 to -1.3) and -4.3 (-9.7 to -0.9) ml/min per 1.73m^2, respectively, but not in group C (GFR decrease -0.7 (-1.1 to 1.5) ml/min/1.73m^2). The percentage change in GFR, ERPF (^{131}I-hippuran clearance), and filtration fraction with tolvaptan did not differ between the three study groups. No differences between the three study groups were found in other main efficacy variables, besides smaller increases in urine volume in group C during tolvaptan treatment. Tolvaptan was well tolerated, with only two patients withdrawing. Thus, doses of tolvaptan typically used in patients with ADPKD do not produce a difference in renal hemodynamic profile in chronic kidney disease stages 1 through 4, but minor GFR drops may be observed in individual patients.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease, with an incidence of 0.25–2 in 1000 newborns [1]. In ADPKD, cysts progressively enlarge and new cysts are formed from birth in both kidneys, eventually resulting in loss of kidney function, with many affected patients requiring renal replacement therapy between their fourth and sixth decade of life [1]. There is no effective treatment currently available to delay disease progression.

Vasopressin is hypothesized to have an important role in the pathogenesis of ADPKD. Vasopressin promotes 3'–5'-cyclic adenosine monophosphate production when bound to vasopressin V2 receptors in the distal nephron and collecting ducts. This leads to differentiated function in normal kidney; however, in epithelial cells that carry abnormal genes, it produces abnormal proliferation and chloride-driven fluid secretion into cysts, thus leading to cyst formation and growth [2,3]. Vasopressin V2-receptor antagonists are a promising potential treatment for ADPKD [4,5]. In rodent models, these agents have been shown to reduce total kidney volume and lower blood urea nitrogen [6-9].

Tolvaptan, an orally effective vasopressin V2-receptor antagonist [10], is currently approved in the United States and Canada for treatment of hyponatremia in euvolemic and hypervolemic states, in the European Union for SIADH (syndrome of inappropriate antidiuretic hormone secretion), and in Japan for the treatment of cardiac edema resistant to diuretics. In these trials, a reversible increase of 0.1 mg/dl of serum creatinine was seen with tolvaptan treatment. In a trial including specifically ADPKD subjects, 1 week of administration of low-dose 45/15 mg/day split-dose tolvaptan in 20 subjects also resulted in an increase in serum creatinine and decrease in glomerular filtration rate (GFR), potentially owing to hemodynamic mechanisms [11]. Recovery of renal function after study drug withdrawal was not studied. In the pivotal, large-scale, multicenter, double-blind, randomized, placebo-controlled trial where tolvaptan slowed ADPKD disease progression (as measured by total kidney volume, renal function decline, and renal pain), tolvaptan was administered in doses between 45/15 and 90/30 mg/day as a split dose [12,13]. In that trial, subjects with relatively intact kidney function (estimated creatinine clearance ≥60 ml/min) have been included, but it is expected that subjects lose kidney function during prolonged follow-up [13]. We therefore felt that it is important to investigate the renal hemodynamic effects of a longer (3 week) treatment course with target therapeutic doses of tolvaptan in subjects with normal kidney function, and also mild-to-moderate and severe kidney function impairment. In addition, we investigated the reversibility of potential effects after withdrawal of study medication.

RESULTS

A total of 29 subjects were included in the three estimated glomerular filtration rate (eGFR) groups (group A, chronic kidney disease (CKD) stages I and II; group B, CKD stage III; group C, CKD stages IV and V). Two subjects did not complete the trial because of an adverse event. These subjects were included in the assessment of the adverse event profile of tolvaptan but could not be included in the analyses of effects on renal hemodynamics, because they withdrew from the study before formal assessment of the effects of tolvaptan at the end of the 3-week treatment period. Thus, 27 subjects completed the study (n=9 per study group). The baseline characteristics are shown in Table 1. As intended by the stratification criteria, subjects in group C had the lowest eGFR, and consequently also the lowest measured GFR and effective renal plasma flow (ERPF). Furthermore, they had the highest median age, blood pressure, highest serum potassium levels, plasma osmolality, plasma copeptin, and 24-h urine volume.

Table 1 Baseline characteristics (median and interquartile ranges unless specified otherwise) per study group (stratified for baseline eGFR)

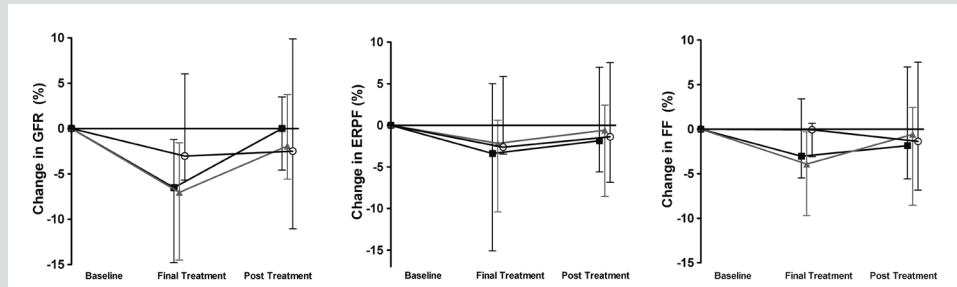
				p-value			
	Group A (N=9)	Group B (N=9)	Group C (N=9)	K-W	A vs B	A vs C	B vs C
eGFR (mL/min/1.73m²)	83 (75 - 95)	47 (39 - 60)	18 (16 - 25)	<0.001	<0.001	<0.001	<0.001
Age (yr)	37 (35 - 45)	47 (38 - 57)	52 (48 - 58)	0.022	0.171	0.004	0.270
Male gender N (%)	4 (44)	3 (33)	7 (78)	0.156			
Weight (kg)	75 (65 – 95)	74 (67 – 96)	98 (80 – 103)	0.217			
BSA (m²)	1.9 (1.8 - 2.2)	2.0 (1.8 - 2.2)	2.3 (2.0 - 2.3)	0.237			
Heart rate (beats/min)	70 (58 - 78)	59 (54 - 71)	68 (57 - 75)	0.499			
MAP (mmHg)	88 (85 - 95)	86 (84 - 92)	94 (89 - 109)	0.061	0.596	0.070	0.031
Antihypertensive drug use N (%)	7 (78)	8 (89)	9 (100)	0.338			
ACEi/ARB use N (%)	7 (78)	8 (89)	8 (89)	0.754			
Serum creatinine (umol/L)	76 (69 – 80)	135 (89 – 143)	280 (242 – 319)	<0.001	0.002	<0.001	<0.001
Serum sodium (mmol/L)	140 (139 – 142)	141 (140 – 143)	140 (140 – 142)	0.481			
Serum potassium (mmol/L)	3.9 (3.9 - 4.0)	4.2 (4.1 - 4.4)	4.5 (4.1 - 4.8)	0.002	0.003	0.003	0.128
Serum uric acid (mmol/L)	0.30 (0.18 – 0.31)	0.34 (0.25 – 0.41)	0.43 (0.42 – 0.47)	<0.001	0.024	<0.001	0.008
Blood urea nitrogen (mmol/L)	5.6 (4.9 - 6.6)	8.2 (5.3 - 10.0)	17.0 (15.4 - 18.9)	<0.001	0.070	<0.001	0.001
Plasma osmolality (mOsm/kg)	281 (280 - 282)	287 (281 - 291)	295 (292 - 299)	<0.001	0.050	<0.001	0.005
Plasma copeptin (pmol/L)	6.4 (3.6 – 7.6)	9.2 (3.8 – 18.5)	30.0 (13.0 – 45.2)	0.001	0.216	<0.001	0.009
Urine volume (ml/24hr)	1720 (1530–2575)	2790 (2358 – 3325)	3050 (1935 – 3200)	0.017	0.012	0.019	0.659
GFR (mL/min/1.73m²)	106 (92 - 110)	61 (41 - 73)	22 (17 - 30)	<0.001	0.001	<0.001	<0.001
ERPF (mL/min/1.73m²)	305 (260 - 341)	207 (151 - 234)	73 (64 - 96)	<0.001	0.002	<0.001	<0.001
FF (GFR/ERPF)	0.34 (0.32 - 0.36)	0.29 (0.28 - 0.31)	0.30 (0.28 - 0.32)	0.014	0.011	0.0013	0.823

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BSA, body surface area; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease (MDRD)); ERPF, effective renal plasma flow; FF, filtration fraction; K–W, Kruskal–Wallis test; MAP, mean arterial pressure. If the K–W test (comparison between three groups) yielded a value of P<0.1, between-group comparisons were performed with the Mann–Whitney U-test.

Figure 1. Percent change compared with baseline in glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and filtration fraction (FF) from baseline, after 3 weeks of treatment with tolvaptan, and 3 weeks after withdrawal. Results for GFR are given in the left panel, for ERPF in the middle panel, and for FF in the right panel. Shown are median changes with interquartile range.

Group A (estimated GFR (eGFR) >60 ml/min per 1.73m²) is represented by black squares, group B (eGFR 30–60 ml/min per 1.73m²) by gray triangles, and group C (<30 ml/min per 1.73m²) by open circles.

All groups consisted of nine subjects, but the post treatment group C consisted of eight subjects, because one subject used diuretics between final treatment and after treatment, which was not allowed as per protocol. No significant differences between groups were found in tolvaptan-induced changes in GFR, ERPF, or FF.



Changes compared with baseline induced by tolvaptan were calculated for the main study parameters GFR, ERPF, and filtration fraction (FF). After 3 weeks of tolvaptan treatment, GFR decreased significantly in the overall study population (median -5.4%, P=0.001), as well as FF (median -2.9%, P=0.02), whereas ERPF did not change significantly (median -2.6%, P=0.10). The results for renal hemodynamic parameters are shown per eGFR study group in Table 2. There was a statistically significant difference in absolute decrease in 'Final treatment GFR' between groups A and C (-7.8 vs. -0.7 ml/min per 1.73m², P=0.02), and between groups B and C (-4.3 vs. -0.7 ml/min per 1.73m², P=0.009), which was not present when changes in GFR were expressed as percentage. The tolvaptan-induced change in GFR was reversible after study drug withdrawal. Changes in ERPF or FF were not different between the three study groups, neither expressed as absolute nor as percentage. Percent changes in renal hemodynamic variables at the end of the tolvaptan treatment period are shown in Figure 1. It is noteworthy that the variability in relative change in GFR was higher in group C compared with groups A and B (Figure 1). On an absolute scale, however, variability in GFR in this group was lower, and was comparable to groups A and B (Supplementary Figure S1 online). The larger relative variation should therefore be interpreted as the consequence of technical issues inherent to the measurement technique and rounding of figures rather than of clinical relevance.

The tolvaptan-induced changes in GFR estimated with the Modification of Diet in Renal Disease (MDRD; and other creatinine- and/or cystatin-based equations) equation were qualitatively similar to the changes in GFR measured as ^{125}I-iothalamate clearance.

Table 2. Change in renal hemodynamic variables after 3 weeks of treatment with tolvaptan (final treatment) and 3 weeks after withdrawal of tolvaptan (after treatment)

Variable	Treatment phase	Absolute change			p-value			
		Group A	Group B	Group C		K-W	A vs B	A vs C	B vs C
GFR	Final treatment	-7.8* (-13.7 - -1.3)	-4.3* (-9.7 - -0.9)	-0.7 (-1.1 – 1.5)	0.016	0.508	0.022	0.009	
	Post treatment	0.0 (-4.3 – 3.8)	-0.8 (-3.3 – 1.8)	-0.4 (-2.7 – 2.2)	0.862				
ERPF	Final treatment	-9.0 (-50.6 – 16.1)	-4.5 (-23.1 – 1.8)	-1.6 (-2.9 – 5.9)	0.237				
	Post treatment	-4.9 (-15.2 – 19.6)	-1.0 (-21.7 – 3.4)	-1.1 (-6.2 – 6.1)	0.735				
FF	Final treatment	-0.01 (-0.02 – 0.01)	-0.01 (-0.03 - -0.01)	0.00 (-0.01 – 0.00)	0.140				
	Post treatment	0.01 (-0.02 – 0.02)	0.01 (-0.01 – 0.01)	-0.01 (-0.02 – 0.00)	0.340				
Variable	**Treatment phase**	**Percentage change**			**p-value**				
		Group A	Group B	Group C		K-W	A vs B	A vs C	B vs C
GFR	Final treatment	-6.5* (-14.8--1.2)	-7.0* (-14.5--1.6)	-3.0 (-5.7 – 6.0)	0.121				
	Post treatment	0.0 (-4.6 – 3.5)	-1.9 (-5.6 – 3.7)	-2.5 (-11.6 – 12.4)	0.929				
ERPF	Final treatment	-3.4 (-15.1 – 5.0)	-2.2 (-10.4 – 0.6)	-2.6 (-3.5 – 5.9)	0.556				
	Post treatment	-1.8 (-5.6 - 7.0)	-0.6 (-8.5 – 2.4)	-1.4 (-8.6 – 9.7)	0.816				
FF	Final treatment	-2.7 (-6.0 – 3.2)	-3.9 (-9.7- -1.7)	0.0 (-3.2 – 0.0)	0.109				
	Post treatment	2.9 (-5.9 – 4.2)	2.9 (-3.3 – 3.5)	-1.7 (-7.1 – 0.0)	0.270				

Abbreviations: ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; K–W, Kruskal–Wallis test.
GFR and ERPF adjusted for body surface area (BSA). The P-values have been calculated with a Kruskal–Wallis test (comparison between three groups), and in cases where P<0.1, between-group comparisons were performed with the Mann–Whitney U-test. All groups consisted of nine subjects, but the post-treatment group C consisted of eight subjects, because one subject used diuretics between final treatment and after treatment, which was not allowed as per protocol. The P-values of paired variables have been calculated by the Wilcoxon signed-ranks test.
*P<0.05 versus baseline. Upper panel shows absolute changes (medians with interquartile ranges) and lower panel shows percent change (medians with interquartile ranges) in the main study parameters.

Figure S1. GFR in individual ADPKD patients at baseline, during tolvaptan treatment and after withdrawal.

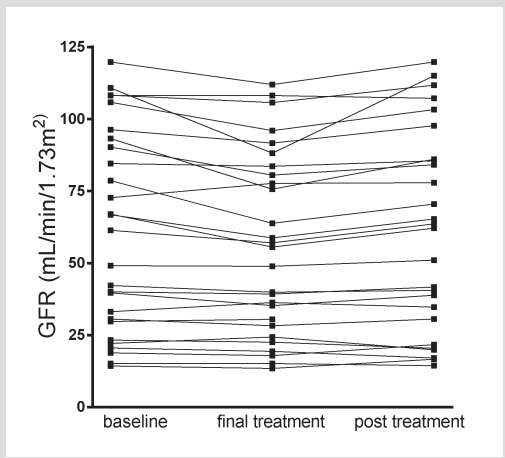


Figure S2. Correlation between measured and estimated (MDRD) GFR.

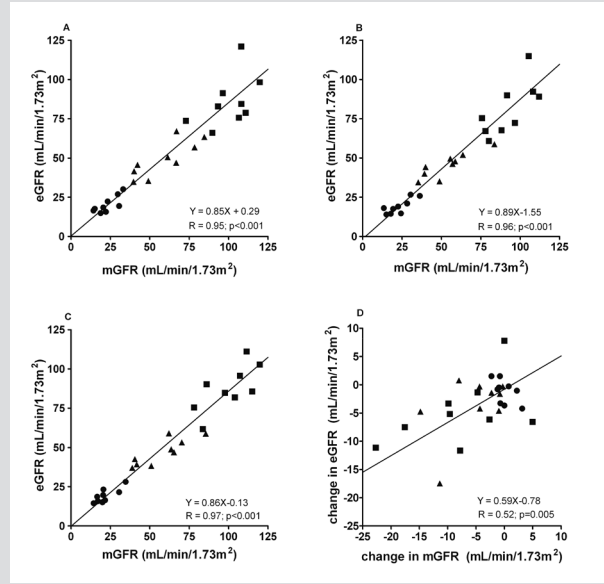


Table S1. Pearson correlation and the slope (β) of the association between measured GFR (^{125}I-iothalamate clearance, adjusted for BSA) and GFR estimated by the MDRD equation[28]; the CKD-EPI equation[34]; the cystatin C equation[35] and the cystatin C combined with creatinine equation[35].

	β	R	p
Baseline, mGFR versus GFR estimated with			
- MDRD	0.85	0.95	<0.001
- CKD-EPI	0.96	0.97	<0.001
- Cystatin C	1.06	0.97	<0.001
- Cystatin C and creatinine	0.99	0.99	<0.001
- Creatinine clearance	1.16	0.99	<0.001
After 3 weeks treatment, mGFR versus GFR estimated with			
- MDRD	0.89	0.96	<0.001
- CKD-EPI	1.01	0.97	<0.001
- Cystatin C	1.09	0.97	<0.001
- Cystatin C and creatinine	1.04	0.99	<0.001
- Creatinine clearance	1.15	0.98	<0.001
3 weeks after last dose, mGFR versus GFR estimated with			
- MDRD eGFR	0.86	0.97	<0.001
- CKD-EPI	0.99	0.97	<0.001
- Cystatin C	1.07	0.97	<0.001
- Cystatin C and creatinine	1.02	0.99	<0.001
- Creatinine clearance	1.12	0.99	<0.001
Treatment induced change in mGFR versus change in GFR estimated with			
- MDRD	0.59	0.52	0.005
- CKD-EPI	0.75	0.56	0.003
- Cystatin C	0.97	0.61	0.001
- Cystatin C and creatinine	0.51	0.68	<0.001
- Creatinine clearance	1.43	0.80	<0.001

Abbreviations: mGFR, measured GFR (Iothalamate clearance); eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease equation; CKD-EPI, chronic kidney disease epidemiology collaboration equation.

Table 3. Percent change (medians with interquartile ranges) in additional main efficacy and safety variables P-value

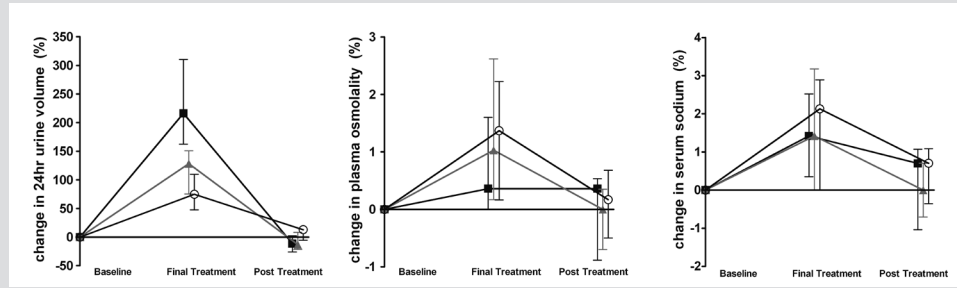
		Group A	Group B	Group C	p-value			
					All	A vs B	A vs C	B vs C
Serum creatinine	Final treatment	7.3* (2.6 - 9.9)	3.4* (0.7 – 7.9)	4.0 (-2.7 – 14.4)	0.772			
	Post treatment	-2.1 (-7.1 – 6.3)	5.2 (-2.6 – 6.5)	4.8 (-8.2 – 13.6)	0.494			
eGFR (MDRD)	Final treatment	-7.8 (-9.2 - -2.9)	-3.8* (-8.4 - -0.8)	-4.4 (-14.4 – 3.4)	0.772			
	Post treatment	4.6 (-6.8 – 8.9)	-5.7 (-7.0 – 3.2)	-5.3 (-13.6 – 10.3)	0.494			
Plasma Cystatin C	Final treatment	4.2* (2.3 – 7.3)	4.7* (2.9 – 5.9)	2.1 (-0.4 – 6.6)	0.526			
	Post treatment	2.3 (-2.7 – 5.0)	0.0 (-1.8 – 4.1)	1.6 (-5.0 – 6.6)	0.975			
eGFR (cystatin C)	Final treatment	-9.0* (-11.7 - -2.0)	-4.4* (-9.6 - -1.0)	-5.1 (-16.4 – 3.9)	0.834			
	Post treatment	2.9 (-5.6 – 4.8)	-6.5 (-8.0 – 3.7)	-6.0 (-15.5 – 12.0)	0.556			
Urine volume	Final treatment	216* (162 - 310)	128* (75 - 151)	75* (48 - 110)	0.001	0.012	0.001	0.122
	Post treatment	-11.3 (-26.0 – 2.3)	-13.1 (-21.5 – 8.4)	13.0 (-5.9 – 17.8)	0.085	0.691	0.027	0.124
Weight	Final treatment	0.0 (-2.7 – 0.5)	-1.5 (-2.0 - -0.3)	-1.2* (-1.9 - -0.4)	0.633			
	Post treatment	1.2* (0.5 – 1.7)	0.6 (-0.6 – 1.5)	0.0 (-1.5 – 1.3)	0.140			
Serum sodium	Final treatment	1.4* (0.4 - 2.5)	1.4* (0.0 - 3.2)	2.1* (0.0 - 2.9)	0.999			
	Post treatment	0.7 (-1.0 – 1.1)	0.0 (-0.7 – 0.7)	0.7 (-0.5 – 1.3)	0.504			
Plasma osmolality	Final treatment	0.4* (0.0 - 1.6)	1.0* (0.2 - 2.6)	1.4* (0.2 - 2.2)	0.770			
	Post treatment	0.4 (-0.9 – 0.5)	0.0 (-0.7 – 0.3)	0.2 (-0.6 – 0.9)	0.831			
MAP	Final treatment	1.5 (-3.9 – 9.9)	-0.4 (-5.4 – 6.5)	-2.5 (-6.3 – 2.1)	0.518			
	Post treatment	1.5 (-2.3 – 8.7)	-3.9 (-4.8 – 5.3)	-2.0 (-6.8 -2.4)	0.302			
Pulse	Final treatment	-5.5 (-13.0 – 3.9)	-1.7 (-8.2 – 3.7)	-3.2 (-6.1 – 13.0)	0.679			
	Post treatment	-2.5 (-10.7 - -0.8)	-2.9 (-13.4 – 2.7)	3.1 (-4.1 – 14.0)	0.149			
Serum uric acid	Final treatment	6.7* (1.6 – 25.6)	16.2* (0.0 – 25.0)	4.7* (0.0 – 10.2)	0.627			
	Post treatment	-6.5 (-17.4 - -0.7)	2.3* (0.0 – 5.9)	-2.4 (-4.6 – 14.1)	0.020	0.015	0.022	0.508
AST	Final treatment	13.6* (6.8 - 27.2)	10.0 (-12.1 – 18.8)	-6.7 (-30.4 – 29.4)	0.375			
	Post treatment	4.5 (-2.2 – 14.9)	-5.9 (-10.3 – 18.6)	-20.8 (-35.6 – 10.5)	0.188			
ALT	Final treatment	21.1 (14.3 - 59.6)	-10.0 (-22.4 – 3.1)	-14.8* (-30.4 - -7.0)	0.002	0.004	0.003	0.289
	Post treatment	0.0 (-18.5 – 27.4)	-9.1 (-18.8 – 17.4)	-21.0 (-49.2 – 6.7)	0.173			
ALP	Final treatment	9.2* (3.2 - 17.3)	0.0 (-4.5 – 5.0)	-5.6 (-8.7 – 5.0)	0.016	0.011	0.019	0.450
	Post treatment	0.0 (0.0 – 8.3)	-7.9* (-13.1 – 0.0)	7.5 (-6.6 – 15.1)	0.027	0.013	0.663	0.034
GGT	Final treatment	9.1 (0.0 - 17.6)	-10.3 (-15.5 - -4.7)	-6.5 (-13.1 – 4.3)	0.039	0.024	0.070	0.233
	Post treatment	0.0 (-6.8 – 6.9)	-9.3 (-20.8 – 10.3)	-5.1 (-16.3 – 22.1)	0.642			
Plasma copeptin	Final treatment	237* (192 – 353)	187* (104 – 413)	94* (42 – 135)	0.006	0.453	0.002	0.023
	Post treatment	-6.2 (-26.6 – 19.9)	3.1 (-19.0 – 21.6)	-14.5 (-40.4 – 12.8)	0.379			
Plasma renin	Final treatment	4.2 (-19.0 – 33.4)	50.0* (-16.9 – 314.7)	-15.3 (-23.3 – 31.1)	0.196			
	Post treatment	-12.2 (-52.8 – 9.7)	0.7 (-31.9 – 34.2)	-9.4 (-36.1 – 45.7)	0.822			
Plasma aldosterone	Final treatment	-11.1 (-22.5 – 67.9)	0.0 (-34.9 – 63.9)	33.3 (-21.7 – 61.5)	0.798			
	Post treatment	40.0 (-35.5 – 158.3)	-20.0 (-38.9 – 20.2)	39.6 (-22.2 – 73.4)	0.265			

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; eGFR, estimated glomerular filtration rate; GGT, g-glutamyl transpeptidase; MAP, mean arterial pressure; MDRD, Modification of Diet in Renal Disease.
All groups consisted of nine subjects, but the post-treatment group C consisted of eight subjects, because one subject used diuretics between final treatment and after treatment, which was not allowed as per protocol.
The P-values have been calculated with the Kruskal–Wallis test (comparison between three groups), and in cases where P00.1, between-group comparisons were performed with the Mann–Whitney U-test (two tailed). P-values of paired variables have been calculated by the Wilcoxon signed-ranks test.
*P<0.05 versus baseline. Values in bold indicate a statistically significant difference between groups.

FIGURE 2 Percentage change compared with baseline in additional key efficacy and safety parameters after 3 weeks of treatment with tolvaptan and 3 weeks after the last dose. Shown are median changes with interquartile range.

Group A (estimated glomerular filtration rate (eGFR) >60 ml/min per 1.73m²) is represented by black squares, group B (eGFR 30–60 ml/min per 1.73m²) by gray triangles, and group C (<30 ml/min per 1.73m²) by open circles.

All groups consisted of nine subjects, but the post-treatment group C consisted of eight subjects, because one subject used diuretics between final treatment and after treatment, which was not allowed as per protocol. Tolvaptan-induced changes in 24-h urinary volume in group A were significantly more than in groups B and C. The changes in plasma osmolality and serum sodium were not significantly different between the groups.



We compared measured GFR with estimated GFR in all subjects (Supplementary Table S1 and Supplementary Figure S2 online). GFR and eGFR were well correlated at baseline (Y=0.85X+0.29, R=0.95, P<0.001) and also at the end of the tolvaptan treatment period (Y=0.89X-1.55, R=0.96, P<0.001) and after withdrawal of the study drug (Y=0.86X-0.13, R=0.97, P<0.001). Tolvaptan-induced changes in GFR and in the MDRD equation eGFR were also correlated, but with lower β (Y=0.59 X-0.78, R=0.52, P=0.005), as were changes in GFR and changes in GFR estimated with the other equations, such as the one with cystatin C, among others (Supplementary Table S1 online).

Table 3 also shows the changes in additional efficacy and safety parameters per group. Tolvaptan induced changes in serum creatinine, uric acid and sodium, plasma osmolality, 24-h urine volume, and plasma copeptin, a surrogate for vasopressin [14,15]. All the changes were showed to be reversible after withdrawal (Figure 2 and Table 3). No differences were observed between the three study groups, besides increases in 24-h urine volume, plasma copeptin, and in some liver function tests (alanine transaminase, alkaline phosphatase, and γ-glutamyl transpeptidase) during tolvaptan treatment. In group C, the changes in these parameters were less when compared with the changes observed in groups A and B. Tolvaptan-induced changes in median weight, serum sodium, and plasma osmolality were not statistically different between the three study groups, but were slightly greater in group C. No significant correlation was found between changes (final treatment vs. baseline) in urinary volume or body weight and changes in serum sodium and plasma osmolality in the overall group or in any of the kidney function groups.

Median maximal plasma concentration (C_{max}) of tolvaptan on the highest tolerated dose was not significantly different between groups (P=0.30) and was 774 ng/ml in group A, 590 ng/ml in group B, and 779 ng/ml in group C. Median T_{max} was 2 h in all study groups. Median $AUC_{0-5\,h}$ (area under the concentration-time curve from time 0 to time of the last measurable concentration) was 2830 h*ng/ml in group A, 1970 h*ng/ml in group B, and 2940 h*ng/ml in group C, with no difference between study groups (P=0.14).

Tolvaptan was generally well tolerated. As mentioned earlier, two subjects did not complete the study: one subject (group A) withdrew from the study because of polyuria after 3 days of treatment on a dose of 45/15 mg, and the other subject (group B) withdrew because of a dry mouth after 13 days of treatment, on a dose of 60/30 mg. Their data are, however, included in the adverse events analysis. One subject (from group A) did not tolerate the 90/30-mg dose owing to polyuria and completed the study on the 60/30 mg dose. All other subjects continued treatment at the highest prescribed dose (split-dose regimen of 90/30 mg). A listing of adverse events (N>1) during tolvaptan treatment is given in Supplementary Table S2 online. The most frequently observed adverse events were thirst, dry mouth, polyuria, and nycturia. No differences in adverse event incidence rate between the three study groups were noted, neither for the overall number of adverse events nor for individual adverse events. The median number of adverse events during tolvaptan treatment was five per subject. One serious adverse event occurred in a subject in group C. This subject was already known to have hypertrophic cardiomyopathy with angina before the study started. His angina worsened from the ninth day of using tolvaptan. He continued the study drug until the final treatment visit. After the last dose of tolvaptan, angina continued. An elective angiography was performed 2 weeks after the last dose of tolvaptan, showing no ischemic heart disease. The reason why angina worsened remains unknown. No relation with the study drug was assumed. This subject was treated with β-adrenergic blocking agents, after which his complaints resolved. Another subject (group C) developed edema after withdrawal of tolvaptan. This subject had a history of edema before the start of the study and used diuretics for this symptom. Before the start of the study, diuretics were withdrawn as per protocol. Edema gradually reappeared during the study, and became clinically relevant shortly after stopping tolvaptan, necessitating reuse of diuretics. Weight at the end of the three study phases was 97.8, 96.6, and 94.8 kg, respectively, and GFR was 29.7, 30.5, and 20.6 ml/min per 1.73m^2, respectively. Because the start of diuretic therapy was expected to influence key study end points, and was not allowed as per protocol, post-treatment data from this subject were not used for renal function analyses.

Table S2. Number of subjects with adverse events. Only treatment emergent events reported for at least two subjects in the study were taken into account (including events reported up to 3 weeks after treatment withdrawal).

	Group		
	A	B	C
Number of subjects	10	10	9
Thirst	10	10	8
Polyuria	10	9	7
Nocturia	8	6	6
Dry mouth	6	5	5
Decreased appetite	2	3	2
Headache	2	2	1
Fatigue	2	1	3
Nausea	1	1	2
Dizziness	1	2	1
Kidney pain	3		
Hot flush	2		1
Dyspnea	1		1
Hypernatremia		1	1
Insomnia			2
Abdominal distension	2		
Abdominal pain	2		
Diarrhea	1	1	

DISCUSSION

In this short-term study performed in 27 subjects with ADPKD stratified in three eGFR study groups, the use of tolvaptan (titrated to 120 mg per day as a 90/30 mg split-dose regimen) had effects on renal hemodynamic parameters, with a decrease in GFR (median <10%) and FF and a nonsignificant decrease in ERPF. No differences in relative renal hemodynamic response to tolvaptan were observed between the three study groups, and in all groups the renal hemodynamic effects were reversible after withdrawal of tolvaptan. Various other safety and efficacy variables were measured. There was a significant rise (final treatment vs. baseline) in serum sodium and plasma osmolality in all three study groups, but there was no significant difference in the changes in these variables between groups. Adverse events were reported in all groups, but they were not significantly more in the low-eGFR group compared with the higher-eGFR groups.

There have been few studies conducted to date that have investigated the renal hemodynamic effects of tolvaptan in ADPKD subjects. These studies were conducted in subjects with near-normal kidney function (CKD stages 1 and 2)[5,12] or mildly impaired kidney function [11] (CKD stage ≤3). It is important to know whether tolvaptan is safe to use in ADPKD subjects with later-stage CKD and what the renal hemodynamic effects are in these subjects. Furthermore, if tolvaptan treatment is initiated at a more normal kidney function and continued as kidney function deteriorates, it should be known whether, and when, treatment should be stopped. Recently, Irazabal et al.[11] reported short-term effects on renal hemodynamics of tolvaptan in ADPKD subjects with eGFR >30 ml/min and found that there was a significant rise in serum creatinine because of a decrease in GFR after

a 1-week use of tolvaptan at a dose of 45/15mg per day. We confirmed these results in this 3-week higher-dose tolvaptan study, and found that these effects were reversible after discontinuation of tolvaptan. In subjects with low eGFR (<30 ml/min per 1.73m²), the absolute renal hemodynamic effects were less explicit compared with ADPKD subjects with higher eGFR. In addition, in our overall study population, FF decreased, whereas ERPF did not change significantly. This renal hemodynamic pattern may suggest preglomerular vasoconstriction and a decrease in intraglomerular pressure, which may be beneficial for preservation of kidney function in the long term. These results should not be interpreted as providing evidence for long-term benefit in subjects with lower GFR. It is noteworthy that the short-term renal hemodynamic effects that we observed in our study are slightly less in group C, and these subjects showed less increase in urinary volume. The long-term effects of tolvaptan on the rate of renal function decline and kidney growth in ADPKD subjects with low GFR need additional investigation.

The cause of the decrease in GFR that we observed during treatment could be explained by several mechanisms. First, it may be related to the aquaretic effect of tolvaptan, resulting in mild dehydration. We found, however, no significant association between the decrease in GFR and decrease in body weight (R=0.30, P=0.12) or the increase in serum sodium concentration (R=0.05, P=0.79). Second, we observed an increase in plasma levels of copeptin, a surrogate of vasopressin,[14,15] during treatment with the V2 receptor antagonist tolvaptan. Vasopressin binds to V1a receptors, which results in vasoconstriction. However, this is expected to occur predominantly in efferent arterioles,[16,17] thus leading to an increase in GFR instead of a decrease. Third, because of high vasopressin levels, the renin–angiotensin–aldosterone system will be activated.[18] Again, this is expected to result in higher intraglomerular pressure and an increase in GFR. In addition, we found no associations between change in GFR and changes in renin (R= -0.08, P=0.70) or aldosterone (R=0.17, P=0.40) concentrations. More likely explanations for the decrease in GFR during tolvaptan use may be that because of less stimulation of the vasopressin V2 receptor, urea recycling will diminish, resulting in a diminution of tubuloglomerular feedback,[19,20] and that higher circulating vasopressin levels may lead to a reduction in the glomerular ultrafiltration coefficient caused by V1a receptor–mediated mesangial cell contraction.[21] Another explanation might be an increase in tubular pressure due to the aquaretic effect of tolvaptan, leading to a reduced net driving force resulting in lower GFR.[22]

Recently, the value of creatinine-based estimation equations to assess GFR in ADPKD has been questioned.[23] It is noteworthy that we found highly significant associations between measured GFR (iothalamate clearance) and GFR estimated with various clinically used estimation equations at baseline, as well as during treatment. Moreover, we found a significant association between change in mGFR and change in GFR estimated using creatinine-based equations, as well as cystatin C–based equations. These observations suggest that creatinine-based GFR estimation equations can be used in studies investigating the effects of tolvaptan in ADPKD.

Serum sodium and osmolality increased in all three eGFR groups, which was expected because of the aquaretic effect of tolvaptan. At baseline, plasma osmolality was higher in group C, whereas serum sodium level was similar in all groups. While using tolvaptan, increases in plasma osmolality and serum sodium were statistically, but not clinically, significant in all groups, with no differences between groups. The highest serum sodium level on treatment was 147 mEq/l. This is only slightly above the reference range, but these data indicate that monitoring serum sodium levels should be recommended in subjects taking tolvaptan and that these subjects should have free access to water in order to prevent dehydration and hypernatremia.

Bioavailability of tolvaptan following a single oral 30-mg dose was reported to be 56% (range 42–80%).[24] Tolvaptan is extensively metabolized by the liver, predominantly via the CYP3A4 pathway,[25] and renal clearance of tolvaptan is <1% of total body clearance.[26] In line with this, we found no differences in median C_{max}, T_{max}, or $AUC_{0-5\ h}$ of tolvaptan between the three strata of kidney function.

A limitation of this study is that only 27 subjects were included, with 9 subjects per study group. However, the a priori performed power analysis indicated that six subjects per study group would be sufficient to study the primary outcome measure, that is, short-term changes in renal hemodynamics. For investigating the potential long-term renoprotective efficacy of tolvaptan in ADPKD subjects with low eGFR (<30 ml/min per 1.73m²), a larger (randomized) study with longer follow-up is needed. It is important to note that for a formal safety analysis the present number of 27 included subjects is too low to draw firm conclusions on the issue of whether tolvaptan can be safely used in subjects with eGFR <30 ml/min per 1.73m².

The strengths of this study are that we measured GFR and ERPF with gold-standard methods (iothalamate and hippuran clearance, respectively) and assessed a large number of safety variables. This was done not only on treatment but also after withdrawal of tolvaptan in order to analyze whether treatment effects would be reversible. This has not been tested in earlier studies.

In summary, it can be concluded that GFR decreased in most subjects while using tolvaptan, and because of this decrease serum creatinine increased. These changes were not statistically significant in subjects with eGFR <30 ml/min per 1.73m², and these effects were reversible after withdrawal of tolvaptan. In addition, both GFR and change in GFR were significantly associated with eGFR and change in eGFR, respectively, suggesting that these estimation equations can be used in ADPKD subjects with or without using tolvaptan.

MATERIALS AND METHODS

Study population

Study participants were diagnosed with ADPKD based on the Ravine criteria.[27] The participants were 18–70 years of age and were assigned by eGFR (MDRD equation[28]) in three groups: >60 (CKD stage I and II; group A), 30–60 (stage III; group B), and <30 (stage IV and V; group C) ml/min per 1.73m^2.

Exclusion criteria were as follows: body mass index >35 kg/m^2, use of diuretics, pregnancy, or breast feeding, allergic reactions on tolvaptan-related structures or iodine, any form of renal replacement therapy, previous exposure to tolvaptan, administration of an investigative drug or blood donation within 30 days before dosing, significant kidney disease other than ADPKD, risk factors for renal impairment other than ADPKD, recent renal surgery, diabetes mellitus, significant coagulation defects or hemorrhagic diathesis, contraindications to magnetic resonance tomography, disorders in thirst recognition, critical electrolyte imbalances, and uncontrolled hypertension.

Participants with hypertension were treated with angiotensin I–converting enzyme inhibitor or an angiotensin II receptor blocker, with the addition of any other antihypertensive drug if needed, except diuretics, because diuretic therapy is expected to influence key study endpoints of this trial.

The study was approved by the Ethical Board at the University Medical Center Groningen and conducted in adherence to the ICHGCP (International Conference on Harmonization-Good Clinical Practice). Written informed consent was obtained from all subjects. The trial was listed on clinicaltrials.gov (NCT01336972).

Study design

Subjects were screened 2–42 days before dosing of tolvaptan. Subjects were instructed to collect urine for 24 h before every kidney function measurement. Subjects were instructed not to drink alcohol or use any food or beverages containing methyl xanthines within 24 h of kidney function testing in order to avoid effects on vasopressin signaling. As tolvaptan is a weak CYP3A4 substrate, subjects were instructed not to use grapefruit or Seville oranges within 72 h before dosing of tolvaptan.

At 1 day before starting tolvaptan, subjects visited the clinic for kidney function measurements (baseline visit). The day after the baseline visit, subjects started tolvaptan treatment in a split-dose regimen with 45 mg in the morning and 15 mg ~8 h after the first dose. After 1 week of treatment, subjects visited the outpatient clinic to report adverse events, and for measurement of blood pressure and weight. Blood and urine samples were collected. In case the low dose was tolerated, subjects started an intermediate dose; 60 mg tolvaptan in the morning and 30 mg ~8 h thereafter (60/30 mg/day split dose). After another week, the subjects visited the clinic again, with the same procedures being

performed, and the dose of study medication was uptitrated to a split-dose regimen with 90/30 mg/day tolvaptan if tolerated. On the last day of treatment, as well as 3 weeks after the last dose of tolvaptan, subjects visited the clinic where kidney function was again measured. On the last day of treatment, the highest tolerated dose of tolvaptan was administered 30 min after the start of kidney function tracer infusion.

Because of the large number of variables measured in this intensive study protocol, this study focuses on the a priori–defined key outcome measures, which are renal hemodynamic changes and safety during tolvaptan use, whereas a second study will report the effects on other variables.[29]

Measurements and calculations

On kidney function measurement days, subjects visited the clinic at ~0745 h, while they were fasting for at least 4 h (but drinking water ad libitum). Blood pressure was assessed with an automatic device (Dinamap, Critikon, Tampa, FL) for 10 min after the start of tracer infusion solution. The mean of the last three blood pressure measurements was used. Mean arterial pressure was calculated using the standard formula 2/3 diastolic blood pressure +1/3 systolic blood pressure. Weight and height were measured before kidney function measurements started. Body surface area was calculated using the Mosteller formula. [30] Blood samples were drawn before the start of infusion solution, in which serum sodium, potassium, urea, glucose, and liver functions were measured using standard methods. Creatinine was measured with the Roche (Basel, Switzerland) enzymatic creatinine assay (isotope dilution mass spectometry traceable). Plasma and urine osmolality was measured using freezing-point depression.

Kidney function measurements used the constant infusion method with ^{125}I-iothalamate to measure GFR and with ^{131}I-hippuran to measure RPF.[31,32] After drawing a time zero blood sample at ~0800 h, a priming solution containing 20 ml of infusion solution (0.04 MBq of ^{125}I-iothalamate and 0.03 MBq of ^{131}I-hippuran) was given, followed by a constant infusion of 6–12ml/h, with the lowest infusion rates in subjects with impaired kidney function on the basis of the serum creatinine at screening. At 1 h after the infusion solution was given, subjects received a standardized light breakfast. Plasma concentrations of both tracers were allowed to stabilize during 1.5 h of equilibration, which was followed by two 2-h periods for simultaneous assessment of clearances of ^{125}I-iothalamate and ^{131}I-hippuran. Clearances were calculated as UxV/P_{iot} and (IxV)/P_{hipp}, respectively. Because urinary clearance of ^{131}I-hippuran equals plasma clearance in case of perfect urine collection, we routinely use the ratio of plasma-to-urinary clearance of ^{131}I-hippuran to correct urinary clearance of ^{125}Iiothalamate for voiding errors. Because extrarenal clearance of ^{131}Ihippuran may become important at very low kidney function, UxV/P_{hipp} was used to assess ERPF in case UxV/P_{hipp} was <100ml/min. This method to correct for voiding errors has been validated and described before in detail.[31-33] GFR and ERPF were corrected for baseline Body surface area. FF was calculated by dividing GFR by ERPF.

Pharmacokinetics

Plasma samples for measurement of tolvaptan concentration were collected at the final treatment visit (highest dose of tolvaptan) at six time points: 0800 h (before the start of kidney function measurement), 0930 h (1 h of taking 90mg of tolvaptan), 1030, 1130, 1230 pm, and 1330 h. Tolvaptan concentration was measured in these samples using a reverse-phase high-performance liquid chromatographic system with tandem mass spectrophotometric detection, as described previously.[26] OPC-411000, an analog containing an additional methyl group, was used as an internal standard. The lower limit of quantification was 5.00 ng/ml.

STATISTICAL ANALYSES

Analyses were performed at the study center with SPSS version 18.0 (SPSS, Chicago, IL). Because of the low numbers per group, all variables were considered to have a nonnormal distribution and are therefore given as median (interquartile range). For all analyses, a two-sided P-value of <0.05 was considered to indicate statistical significance.

A power analysis was performed to determine how many participants were to be included in this study. Intra-subject day-today coefficient of variation in GFR measurement applying the continuous infusion method of radioactive isotopes is 2.2%, and for ERPF 5.0%. [28,31] The α-level was set at 0.05 and the β-level at 95% (instead of 80%) to decrease the chance of a false-negative finding. To detect a change in GFR or ERPF of 10% (which was chosen as representing a clinically significant change), at least six subjects per study group were needed to complete this study.

Baseline characteristics are presented by group based on the eGFR value obtained during the screening visit. Differences between groups were calculated with the Kruskal–Wallis test (comparison between three groups), and in cases of P<0.1, between-group comparisons were performed with the Mann–Whitney U-test. For comparisons within one group (for example, baseline vs. treatment), the Wilcoxon signed-ranks test was used.

The primary outcome measure of the trial was the change from baseline in measured GFR (as determined by iothalamate clearance), ERPF (as determined by hippuran clearance), and FF (GFR/ERPF). The effects of tolvaptan on these renal hemodynamic parameters were assessed, as was the reversibility of these effects after withdrawal of study medication. As in clinical practice kidney function is estimated with an equation, we also assessed GFR estimated with creatinine-based equations: the MDRD,[28] CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration),[34] and cystatin C–based equations (that is, CKD-EPI with cystatin C and the CKD-EPI equation with cystatin C and creatinine combined).[35] The correlation between measured GFR and GFR estimated with these equations was assessed at the end of each of the three study periods using orthogonal regression analysis (for regression coefficients) and Pearson's correlation analysis (for R

and P-values). We also investigated the correlation between tolvaptan-induced changes in measured and estimated GFR.

Secondary end points included change from baseline pharmacokinetic parameters (maximal (peak) plasma concentration (C_{max}), time to C_{max} (T_{max}), and AUC_t of tolvaptan in plasma.

Other pharmacodynamic and safety parameters were investigated, including change in urine volume, serum sodium and plasma osmolality, weight, pulse, blood pressure, serum creatinine, and standard serum chemistry and hematology tests between groups during the use and after withdrawal of tolvaptan. Differences between groups were similarly tested using Kruskal–Wallis and Mann–Whitney U-tests.

DISCLOSURE

FSC, DO, SES, and HBK are employees of Otsuka. RTG is a member of the steering committee for the Otsuka TEMPO 3:4 trial [12] and is a consultant for Otsuka. JS is an employee of ThermoFisher Scientific, B.R.A.H.M.S. Biomarkers, the company that manufactures and holds patent rights on the copeptin assay. All the other authors declared no competing interests.

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8A

ASSOCIATION OF URINARY BIOMARKERS WITH DISEASE SEVERITY IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE: A CROSS-SECTIONAL ANALYSIS

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ABSTRACT

Disease monitoring of ADPKD will become more important with upcoming therapeutic potential interventions. Since serum creatinine is considered of limited use and measurement of effective renal blood flow (ERBF) and total renal volume (TRV) are time consuming and expensive, there is a need for other biomarkers. We aimed to investigate which urinary markers are elevated in ADPKD, whether these urinary markers are associated with measured glomerular filtration rate (mGFR), ERBF and TRV and whether these associations are independent of albuminuria (UAE).

We measured 24-h urinary excretion of albumin, of glomerular- (IgG), proximal tubular- (KIM1, NAG, NGAL, β2 microglobulin) and distal tubular (H-FABP) damage markers and inflammatory markers (MCP-1 and MIF) in 102 ADPKD patients and compared these to 102 age- and gender matched healthy controls. For ADPKD patients, disease severity was assessed by measures of function (mGFR and ERBF, measured by clearance of ^{125}I-Iothalamate and ^{131}I-Hippuran, respectively, during continuous infusion) and structure (TRV, measured by MRI.

In 102 ADPKD patients (aged 40 ±11y, 58% male), all measured urinary biomarkers were elevated compared to healthy controls. Excretion of IgG and albumin were relatively most elevated. ERBF and mGFR were associated with urinary excretion of β2 microglobulin, NGAL and H-FABP, independent of UAE, while TRV was associated with KIM-1, NGAL and MCP-1 independent of UAE.

Our results suggest that markers for multiple parts of the nephron are elevated in ADPKD and that measurement of urinary β2 microglobulin, H-FABP, MCP-1 and especially NGAL could be of value in addition to UAE for determination of disease severity in ADPKD.

INTRODUCTION

Autosomal Dominant Polycystic Kidney Disease (ADPKD), the most common hereditary kidney disease is characterised by progressive cyst formation in the kidneys, leading to pain, hematuria and end stage renal disease. End stage renal disease usually occurs in the 4th-7th decade of life.[1] The clinical course of ADPKD is highly variable between families, but also within families.[2]

Current treatment cannot prevent renal failure.[3;4] However, a better understanding of the pathophysiology of the disease identified promising candidate drugs for renal preservation.[5] Clinical trials have been initiated for vasopressin-V2 receptor antagonists, long-acting somatostatin analogues and mTOR inhibitors.[6] With these upcoming therapeutic regimens, and the existing variation in disease course, monitoring disease severity will become more important.

Measurement of serum creatinine to assess estimated glomerular filtration rate (eGFR) is of limited use in determining disease severity in ADPKD, since it has been suggested that GFR remains relatively long stable due to compensatory hyperfiltration, while disease progresses.[7] Effective renal blood flow (ERBF) and total kidney volume have been proposed as better markers for disease severity, for they predict renal decline during follow-up[7–10] However, measurement of RBF and total kidney volume is time consuming and expensive. Therefore, interest has grown in urinary biomarkers. These biomarkers are relatively easy to obtain and inexpensive to measure. In renal diseases in general, urinary Kidney Injury Molecule-1 (KIM-1) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) for example are well described markers and predictors of renal disease progression [11–13] and may be promising candidate markers in ADPKD. In ADPKD patients, a limited number of urinary biomarkers have been investigated, namely albuminuria, β2 microglobulin,[14;15] NGAL[16] and Monocyte Chemotactic Protein-1 (MCP-1).[17;18] In general, the promising results obtained for these markers have not been corroborated by others, except for albuminuria. Several studies showed that in ADPKD, albuminuria is associated with increased renal volume,[19;20] mean arterial blood pressure, filtration fraction,[20] renal growth and slope of glomerular filtration rate.[9] Albuminuria is therefore a valuable biomarker indicating disease severity and predicting outcome. As such it has become a secondary endpoint in intervention studies in ADPKD [21].

In normal physiology, albumin passes the glomerular filtration barrier only in trace amounts, after which it is reabsorbed in the proximal tubule.[22;23] In general, albuminuria is assumed to reflect predominantly glomerular endothelial damage. ADPKD however is a disease characterized predominantly by structural abnormalities of especially the distal tubules and collecting ducts, and by interstitial inflammation and fibrosis. In ADPKD, albuminuria might therefore also reflect (inflammatory) tubular damage.

Given this background, we investigated in a cross-sectional study, in a well phenotyped cohort of 102 ADPKD patients, (first) whether urinary biomarkers reflecting glomerular,

proximal or distal tubular damage or inflammation are elevated when compared to healthy controls, (second) whether these elevated biomarkers are associated with current markers of disease severity in ADPKD (effective renal blood flow and glomerular filtration rate as functional measures and total renal volume as measure of structural change) and (third) whether these associations are independent of albuminuria.

MATERIALS AND METHODS

Patients and healthy controls

One hundred and eighteen consecutive patients with ADPKD visiting our out-patient clinic meeting our in- and exclusion criteria were asked to participate. Diagnosis of ADPKD was made upon Ravine criteria.[24] Subjects were considered ineligible to participate if they received renal replacement therapy (including renal transplantation), had undergone renal surgery, were unable to undergo magnetic resonance imaging (as having distorting foreign bodies or aneurysmal clips), had other systemic diseases potentially affecting renal function (such as diabetes mellitus), had other specific medical conditions such as pregnancy, lactation, or who were less than 6 months postpartum. After screening, subjects underwent an extensive medical history assessment and were scheduled for a 1-day outpatient clinic evaluation.

For this study, healthy controls were also invited to participate. These control subjects were matched for age and gender and were considered healthy in case they had a history without cardiovascular and/or renal disease, used no medication, had a normal blood pressure (systolic blood pressure < 140 and diastolic blood pressure <90 mmHg) and a normal estimated glomerular filtration rate (>60 ml/min *1.73 m^2). This study was performed in adherence to the declaration of Helsinki. All subjects gave written informed consent.

Measurements and storage of urinary and plasma samples

Blood pressure was assessed with an automatic device (Dinamap) for 15 minutes during the renal function measurement. Systolic- and diastolic blood pressure values were used to calculate mean arterial pressure (MAP) using the standard formula: 2/3 diastolic blood pressure + 1/3 systolic blood pressure. Weight and height were determined. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in square meters.

Patients collected a 24-h urine sample prior to the outpatient visit. Urinary albumin concentration was determined by immunonephelometry (BNII; Dade Behring Diagnostics, www.dadebehring.com). Urine was stored at -80°C until measurement of Immunoglobulin G (IgG), Kidney Injury Molecule 1 (KIM1), N-acetyl-β-D-glucosaminidase (NAG), Neutrophil Gelatinase-Associated Lipocalin (NGAL), β2 microglobulin, Heart-type Fatty Acid Binding Protein (H-FABP), macrophage migration inhibitory factor (MIF) and

monocyte chemotactic protein-1 (MCP-1). Urinary IgG is a marker for glomerular damage, urinary β2 microglobulin, KIM, NAG and NGAL are markers for damage of the proximal tubule,[12;25;26] whereas urinary H-FABP is a damage marker of the distal tubule.[27;28] Urinary MIF and MCP-1 are markers for inflammation.[29–32] Urinary IgG, H-FABP (Hytest, www.hytest.fi), β2 microglobulin (Anogen, www.yesbiotech.com), KIM1, NGAL, MCP-1 and MIF (R&D systems, www.rndsystems.com) were measured by ELISA. Before measurement, urine samples were diluted two times for KIM-1, β2 microglobulin, MCP1 and MIF, 5 times for H-FABP, and 100 times for NGAL and IgG.

Detection limit for KIM-1 was 0.087 ng/ml, for IgG 220 ng/ml, for NAG 22 ng/ml, for MCP 0.04 ng/ml, for MIF 0.06 ng/ml, for β2 microglobulin 18 ng/ml and for H-FABP 0.38 ng/ml. Urinary N-acetyl-β-D-glucosaminidase (NAG) was measured using a modified enzyme assay according to Lockwood and corrected for nonspecific conversion (HaemoScan, www.haemoscan.com). For all urinary markers, urinary excretion was calculated by multiplication with the volume of the 24-hour urine collection, resulting in biomarker excretion expressed per 24 hours.

Prior to renal function measurement, blood samples were drawn for determination of haemoglobin, glucose, creatinine and urea. Concentrations of haemoglobin and glucose were measured in serum using standard methods. Creatinine was measured with the Roche enzymatic creatinine assay (IDMS traceable). Creatinine values were used to calculate an estimated glomerular filtration rate (eGFR), using the 4-variable MDRD formula. Urinary creatinine excretion was normalized to body weight by dividing the 24h urinary creatinine excretion by the patient's weight in kilograms.

Renal function measurements were performed using the constant infusion method with ^{125}I-Iothalamate and ^{131}I-hippuran.[34–37] Effective renal blood flow (ERBF) was calculated as ERPF / (1-Hct). (Hematocrit was measured halfway during the renal function measurement.) Patients underwent a standardized abdominal magnetic resonance imaging protocol without the use of i.v. contrast. Scanning was performed on a 1.5 Tesla MRI Magnetom Avento (Siemens, Erlangen, Germany) with the use of body matrix and spine matrix coils. Renal volume was measured on T2 weighted coronal images[38] (slice-thickness 4.0 mm) using Analyze Direct 8.0 (AnalyzeDirect, Inc., Overland Park, KS) software.

STATISTICAL ANALYSES

Analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL). Normally distributed variables are expressed as mean ± standard deviation (SD), whereas non-normally distributed variables are given as median (interquartile range). A two sided $p < 0.05$ was considered to indicate statistical significance.

Differences between ADPKD patients and healthy controls were tested using the two-sample T-test when normally distributed, or Mann-Whitney test when not normally distributed. Secondary, we compared biomarker excretions for ADPKD patients with a relatively well preserved renal function (eGFR >60) with healthy controls.

To investigate whether biomarker excretions correlated with mGFR, ERBF and total renal volume, Spearman correlation coefficients were calculated. We also analyzed the reference test as dichotomous variables to calculate Receiver Operating Characteristics curves. As cut-off values an ERBF <500 ml/min, an mGFR <60 ml/min and a TRV > 1000 mL were used. To be able to adjust for potential confounders, multiple regression analysis was performed. Biomarkers were log base 2 transformed to fulfil the requirement of normal distribution of the residuals. Two models were built. First, the association between biomarkers and variables of interest was investigated adjusted for age and gender, and second, albuminuria was entered as an independent variable in addition to the previous variables to investigate whether the associations were independent of albuminuria. To check for co-linearity, variance inflation factor diagnostic was used. The VIF diagnostic is the reciprocal of tolerance (1-R squared for the regression of that variable on all the other independents, ignoring the dependent). So when VIF is high, there is high multicollinearity and instability of the beta coefficients. To visualise the independency of albuminuria in the associations of urinary biomarkers with glomerular filtration rate, renal blood flow and total renal volume, figures were made. For these figures, the excretion of various biomarkers was divided into tertiles. Linear regression analysis was used to calculate adjusted values of measured glomerular filtration rate and effective renal blood flow and geometric mean total renal volume (all ± 95% CI of the mean) per tertile. Means were adjusted for age, gender and urinary albumin excretion. P values for the unadjusted tertiles are calculated using ANOVA. As sensitivity analyses, we attempted to adjust for potential errors in 24h urine collection all analyses by repeating all analyses using biomarker/creatinine ratios as the index test (instead of 24h urinary excretions). We also repeated the analyses in which we only included ADPKD subjects with an mGFR > 60 mL/min.

RESULTS

Thirteen patients refused to participate, two patients were not eligible to participate and 1 patient did not collect 24-h urine, leaving 102 patients for analyses. Characteristics of the participating patients and healthy controls are presented in table 1. A total of 102 ADPKD patients (58% male, aged 40 ±11 years) and 102 healthy controls were analysed. As shown in table 1, ADPKD patients had a higher body mass index, blood pressure (despite more frequent use of antihypertensive medication), serum creatinine, and 24-h urinary volume than age-and gender matched healthy controls. Haemoglobin and estimated glomerular filtration rate were lower than in healthy controls.

Table 1. Characteristics of 102 ADPKD patients and 102 age- and gender matched healthy controls.

Variable	ADPKD	Healthy controls	p-value
Age (y)	40 ± 11	39 ± 12	0.5
Male, n (%)	59 (58)	59 (58)	1.0
BMI (kg/ m²)	26±5	23±3	<0.001
BSA (m²)	2.1±0.3	1.9 ± 0.2	<0.001
SBP (mm Hg)	129 ± 12	122 ± 12	<0.001
DBP (mm Hg)	80 ± 9	72 ± 8	<0.001
Antihypertensive medication, n (%)	78 (77)	0 (0)	<0.001
Hb (g/l)	135 ± 14	140 ± 13	0.003
Plasma creatinine (mg/dl)	1.3 ± 0.8	0.8 ± 0.1	<0.001
24h urinary volume (l/24h)	2.3 ± 0.8	2.0 ± 0.8	0.003
eGFR(ml/min per1.73 m²)	68 ± 27	92 ± 12	<0.001
mGFR (ml/min)	91 ± 36	-	-
mGFR/BSA (ml/min/1.73 m²)	77 ± 31	-	-
RBF (ml/min)	498 ± 207	-	-
Total renal volume (l)	1.5 (0.9-2.2)	-	-

Variables are presented as mean ± SD. Significance was tested using the two-sample T-test. Abbreviations are: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hb haemoglobin; eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate; RBF, renal blood flow.

Figure 1. Urinary biomarkers for ADPKD patients and healthy controls. Boxplots with whiskers from minimum to maximum. * indicates p<0.05

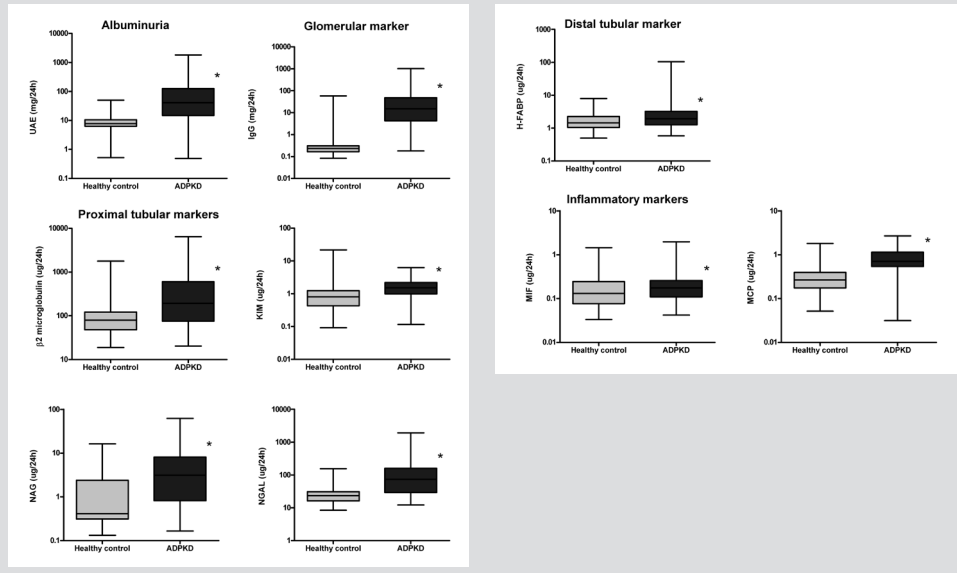


Table 2. Urinary biomarker excretions for ADPKD patients and controls

Variable	ADPKD	Controls	p-value
UAE (mg/24u)	41.2 (14.9-121.7)	7.8 (6.4-10.4)	<0.001
Glomerular			
IgG (mg/24u)	15.3 (4.3-47.5)	0.2 (0.2-0.3)	<0.001
Proximal Tubular			
β2 MG (µg/24u)	192.1 (77.6-589.1)	79.4 (49.1-121.3)	<0.001
KIM-1 (µg/24u)	1.5 (1.0-2.2)	0.8 (0.4-1.2)	<0.001
NAG (U/24u)	3.1 (0.8-8.1)	0.4 (0.3-2.4)	<0.001
NGAL (µg/24u)	73.7 (29.2-158.2)	23.4 (16.5-30.8)	<0.001
Distal Tubular			
H-FABP (µg/24u)	1.9 (1.3-3.2)	1.4 (1.0-2.2)	0.003
Inflammatory			
MIF (µg/24u)	0.2 (0.1-0.3)	0.1 (0.1-0.2)	0.03
MCP (µg/24u)	0.7 (0.6-1.1)	0.3 (0.2-0.4)	<0.001

Variables are presented as median (25th percentile-75th percentile). Significance was tested using the Mann-Whitney U test. Abbreviations are: UAE, urinary albumin excretion; IgG, immunoglobulin G; β2 MG, β2 microglobulin; KIM-1, Kidney Injury Molecule 1; NAG, N-acetyl-β-D-glucosaminidase; NGAL, Neutrophil Gelatinase-Associated Lipocalin; H-FABP, Heart-type Fatty Acid Binding Protein; MIF, macrophage migration inhibitory factor; MCP-1, monocyte chemotactic protein-1.

Association of urinary biomarkers with ADPKD

To answer our first research question, we investigated urinary biomarkers for different segments of the nephron in ADPKD patients and in healthy controls. Urinary creatinine excretion normalized to body weight was 19.3 ± 5.6 mg/kg for the whole group. Table 2 shows that albuminuria, glomerular, proximal- and distal tubular damage markers, as well as markers reflecting inflammation, were all significantly elevated in ADPKD patients compared to healthy controls. IgG excretion and albuminuria were relatively more elevated (6-77 x) in the ADPKD patients than excretion of the other markers (1.4-7.7 x). Values of the urinary markers for ADPKD patients and healthy controls are depicted in box plots in Figure 1. Although significantly different, there is some overlap in biomarker excretions between the ADPKD patients and healthy controls. To adjust for potential errors in 24h urine collection, also marker/creatinine ratios were compared. All urinary biomarker/creatinine ratio's, except MIF, remained significantly higher in ADPKD patients compared to healthy controls. When patients with a reasonably well preserved renal function (eGFR >60 ml/min per 1.73 m², n=60) were selected, all urinary markers (except for H-FABP) were still significantly elevated compared to healthy controls. Also, when patients were selected with a relatively well preserved renal blood flow (ERBF>500 ml/min, n=45) or with a relatively modest total renal volume (TRV <1000 ml, n=30), all urinary markers (except for H-FABP and MIF) were elevated compared to healthy controls.

Figure 2. Associations between urinary biomarkers and Glomerular Filtration Rate.

Depicted are graphs of the urinary biomarkers under study that were significantly associated with mGFR (table 3).

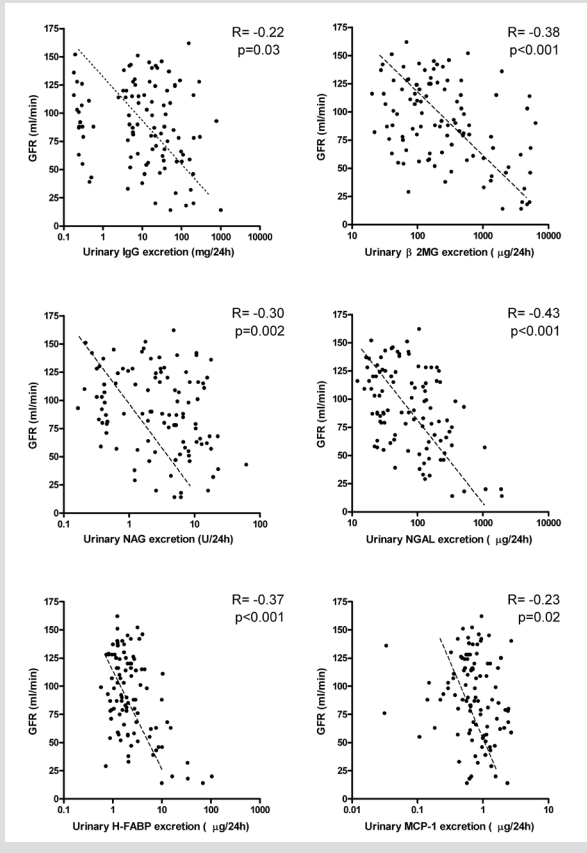


Table 3. Crude correlation coefficients of urinary biomarkers with glomerular filtration rate, renal blood flow and total renal volume in ADPKD patients.

Variable	Glomerular Filtration Rate		Renal Blood Flow		Total Renal Volume	
	R	p-value	R	p-value	R	p-value
Glomerular						
IgG	-0.22	0.03	-0.21	0.04	0.35	<0.001
Proximal Tubular						
β2 MG	-0.38	<0.001	-0.37	<0.001	0.14	0.2
KIM-1	0.09	0.4	0.05	0.6	0.23	0.02
NAG	-0.30	0.002	-0.29	0.004	0.27	0.007
NGAL	-0.43	<0.001	-0.45	<0.001	0.16	0.1
Distal Tubular						
H-FABP	-0.37	<0.001	-0.36	<0.001	0.13	0.2
Inflammatory						
MIF	0.04	0.7	0.03	0.8	0.03	0.8
MCP-1	-0.23	0.02	-0.20	0.05	0.58	<0.001

Correlations and significance were calculated using the spearman correlation coefficient. Abbreviations are: IgG, immunoglobulin G; β2 MG, β2 microglobulin; KIM-1, Kidney Injury Molecule 1; NAG, N-acetyl-β-D-glucosaminidase; NGAL, Neutrophil Gelatinase-Associated Lipocalin; H-FABP, Heart-type Fatty Acid Binding Protein; MIF, macrophage migration inhibitory factor; MCP-1, monocyte chemotactic protein-1.

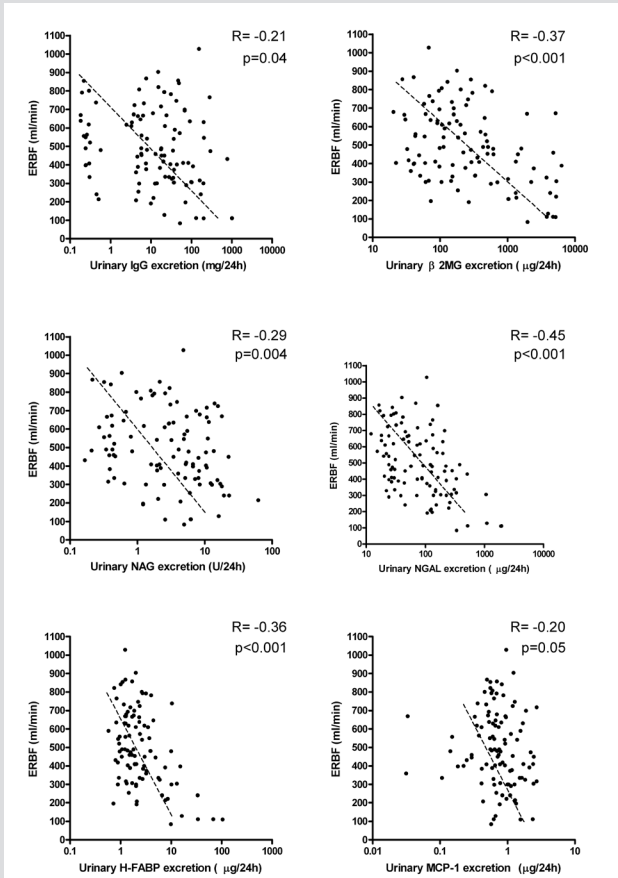


Figure 3. Associations between urinary biomarkers and Effective Renal Blood Flow (ERBF). Depicted are graphs of the urinary biomarkers under study that were significantly associated with ERBF (table 3).

Association of urinary biomarkers with functional measures in ADPKD

Glomerular filtration rate and effective renal blood flow correlated strongly with each other (R=0.94, p<0.001). The associations of urinary markers with measured glomerular filtration rate (mGFR) and effective renal blood flow are depicted in table 3. Figures 2 and 3 depict scatterplots of the associations in table 3 with a p-value below 0.05. These are scatterplots for excretion of IgG, β2 microglobulin, NAG, NGAL, H-FABP and MCP-1 with mGFR (Figure 2), and IgG, β2 microglobulin, NAG, NGAL, H-FABP and MCP-1 with ERBF (Figure 3). For measured glomerular filtration rate and effective renal blood flow, the strongest association was with NGAL. Furthermore, IgG correlated strongly with UAE (R=0.70, p<0.001, not depicted in the table).

ROC curves for an ERBF < 500 ml/min and an mGFR <60 ml/min are depicted in Figure 4 for the urinary biomarkers with the highest R values. All areas under the curves of the depicted urinary biomarkers are significantly different from chance. For both functional measures, NGAL has the largest AUC (0.74 and 0.75 respectively).

Table 4. Multivariable associations of various biomarkers with glomerular filtration rate after adjustment for age, gender (model 1) and additional adjustment for UAE (model 2).

	model	Urinary biomarker			UAE		
		β	95% CI for β	p-value	β	95% CI for β	p-value
UAE	1	-	-	-	-6.1	-8.9- -3.2	<0.001
Glomerular							
IgG	1	-3.2	-5.0- -1.5	<0.001	-	-	-
	2	-1.4	-3.7-0.9	0.22	-4.5	-8.3 - -0.8	0.02
Proximal tubular							
KIM-1	1	2.0	-3.6-7.6	0.48	-	-	-
	2	5.2	-0.07-10.5	0.053	-6.8	-9.7—3.9	<0.001
B2MG	1	-6.0	-8.4 - -3.6	<0.001	-	-	-
	2	-4.8	-7.2- -2.3	<0.001	-4.2	-7.1- -1.4	0.004
NAG	1	-3.3	-6.4- -0.2	0.04	-	-	-
	2	-1.5	-4.5-1.6	0.35	-5.6	-8.6- -2.6	<0.001
NGAL	1	-10.6	-14.0- -7.3	<0.001	-	-	-
	2	-8.9	-12.7- -5.2	<0.001	-2.8	-5.7-0.2	0.07
Distal tubular							
H-FABP	1	-9.3	-13.5- -5.1	<0.001	-	-	-
	2	-7.3	-11.5- -3.1	0.001	-4.6	-7.6- -1.6	0.003
Inflammatory							
MIF	1	-0.1	-5.4-5.1	0.9	-	-	-
	2	2.3	-2.6-7.3	0.4	-6.4	-9.3- -3.5	<0.001
MCP-1	1	-7.5	-12.6- -2.5	0.004	-	-	-
	2	-2.6	-8.4-3.2	0.4	-5.2	-8.6- -1.8	0.003

Beta's, confidence intervals and p-values were calculated using multivariable linear regression. Dependent variable is mGFR, independent variables are the log base2 transformed 24h excretions of the various urinary biomarkers.
Model 1: adjusted for age and gender
Model 2: adjusted for age, gender and albuminuria.
Abbreviations are: IgG, immunoglobulin G; β2 MG, β2 microglobulin; KIM-1, Kidney Injury Molecule 1; NAG, N-acetyl-β-D-glucosaminidase; NGAL, Neutrophil Gelatinase-Associated Lipocalin; H-FABP, Heart-type Fatty Acid Binding Protein; MIF, macrophage migration inhibitory factor; MCP-1, monocyte chemotactic protein-1.

Our third research question was whether these potential associations were independent of albuminuria (urinary albumin excretion, UAE). Table 4 shows the associations between 24h urinary biomarker excretions with measured glomerular filtration rate and Table 5 with effective renal blood flow, both adjusted for age, gender and albuminuria. The urinary biomarker excretions are log base 2 transformed. The beta therefore represents the change in mGFR or RBF per doubling of the biomarker. Of note, albuminuria (adjusted for age and gender) correlated well with both measured glomerular filtration rate and with effective renal blood flow. There are several associations that remained significant after adjustment for age, gender and albuminuria. For both measured glomerular filtration rate and for effective renal blood flow, β2 microglobulin, NGAL and H-FABP remained significantly associated. Figures 5 and 6 depict the associations between biomarkers that were associated with measured glomerular filtration rate independent of albuminuria

Table 5. Multivariable associations of various biomarkers with effective renal blood flow after adjustment for age, gender (model 1) and additional adjustment for UAE (model 2).

	model	Urinary biomarker			UAE		
		β	95% CI for β	p-value	β	95% CI for β	p-value
UAE	1	-	-	-	-30	-47- -13	0.001
Glomerular							
IgG	1	-17	-27- -7	0.001	-	-	-
	2	-9	-23-4	0.18	-20	-42-2	0.08
Proximal tubular							
KIM-1	1	4	-28-37	0.8	-	-	-
	2	19	-12-51	0.2	-32	-50- -15	<0.001
B2MG	1	-34	-48 - -20	<0.001	-	-	-
	2	-28	-43- -14	<0.001	-19	-35- -2	0.03
NAG	1	-21	-39- -3	0.02	-	-	-
	2	-13	-31-5	0.17	-26	-43- -8	0.005
NGAL	1	-55	-75- -35	<0.001	-	-	-
	2	-47	-70- -24	<0.001	-12	-30-6	0.18
Distal tubular							
H-FABP	1	-52	-77- -28	<0.001	-	-	-
	2	-43	-68- -18	0.001	-22	-39- -4	0.02
Inflammatory							
MIF	1	-3	-33-28	0.9	-	-	-
	2	9	-20-39	0.5	-31	-48- -13	0.001
MCP-1	1	-35	-65- -6	0.02	-	-	-
	2	-11	-45-23	0.5	-26	-46- -6	0.01

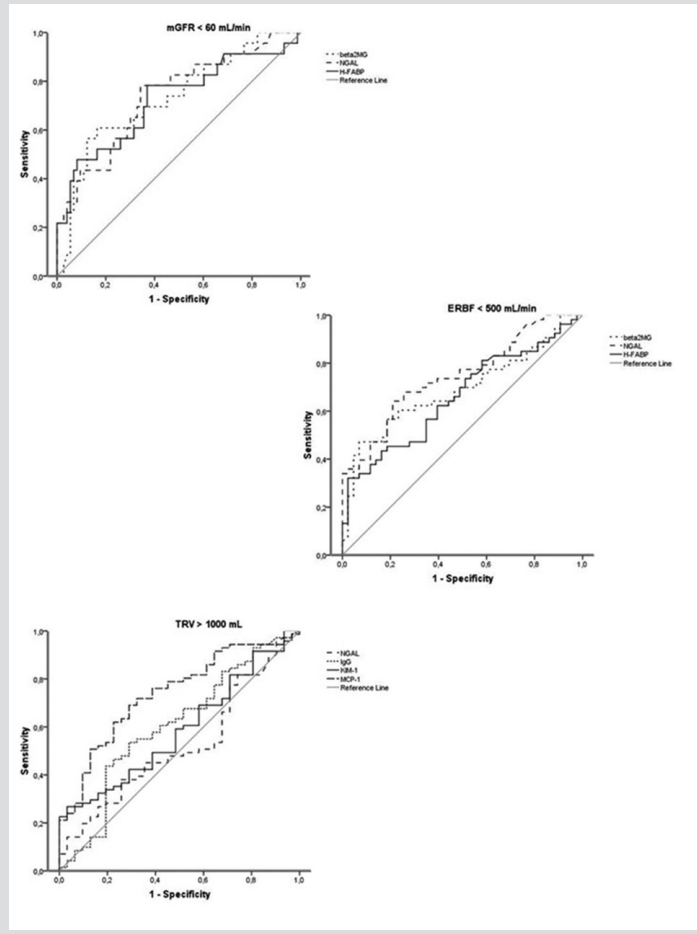
Beta's, confidence intervals and p-values were calculated using multivariable linear regression.
Dependent variable is ERBF, independent variables are the log base2 transformed 24h excretions of the various urinary biomarkers.
Model 1: adjusted for age and gender
Model 2: adjusted for age, gender and albuminuria.
Abbreviations are: IgG, immunoglobulin G; β2 MG, β2 microglobulin; KIM-1, Kidney Injury Molecule 1; NAG, N-acetyl-β-D-glucosaminidase; NGAL, Neutrophil Gelatinase-Associated Lipocalin; H-FABP, Heart-type Fatty Acid Binding Protein; MIF, macrophage migration inhibitory factor; MCP-1, monocyte chemotactic protein-1.

(Figure 5) and effective renal blood flow independent of albuminuria (Figure 6). The figures show mean values of measured glomerular filtration rate and effective renal blood flow for tertiles of the aforementioned biomarkers, both crude and after adjustment of age, gender and albuminuria.

Association of urinary biomarkers with structural changes in ADPKD

Effective renal blood flow (ERBF) and glomerular filtration rate (mGFR) correlated with total renal volume (TRV) (R=-0.20, p=0.05 and R=-0.28, p=0.004 respectively). Crude associations between the urinary biomarker excretions and total renal volume are depicted in table 3. Figure 7 consists of 4 scatterplots showing the association between IgG, KIM-1, NAG and MCP-1 with TRV. The strongest association for total renal volume was

Figure 4. Receiver Operating Characteristics curves for various urinary biomarkers for dichotomized mGFR (cut-off <60 ml/min), ERBF (cut-off 500 ml/min) and TRV (cut-off >1000 mL).



with MCP-1 excretion. The ROC curve in Figure 4 for a total renal volume < 1000 mL shows that MCP-1 has the largest AUC (0.73).

To investigate whether the potential associations were independent of albuminuria (urinary albumin excretion, UAE), Table 6 shows the associations between 24h urinary biomarker excretions and total renal volume adjusted for age, gender and albuminuria. KIM-1, NGAL and MCP-1 remained associated with total renal volume. Figure 8 depicts the associations between the biomarkers that were associated with total renal volume independent of albuminuria. The figures show geometric mean values of total renal volume for tertiles of the aforementioned biomarkers, both crude and after adjustment of age, gender and albuminuria. Thus, NGAL remained associated with measured GFR, effective renal blood flow and total renal volume; all other biomarkers are associated with either mGFR / effective renal blood flow or total renal volume.

Figure 5. The associations between β2 microglobulin (upper panel), NGAL (middle panel) and H-FABP (lower panel), and mGFR are independent of albuminuria. Tertiles of the biomarkers are shown crude (light grey) and after adjustment of age, gender and albuminuria (dark grey).

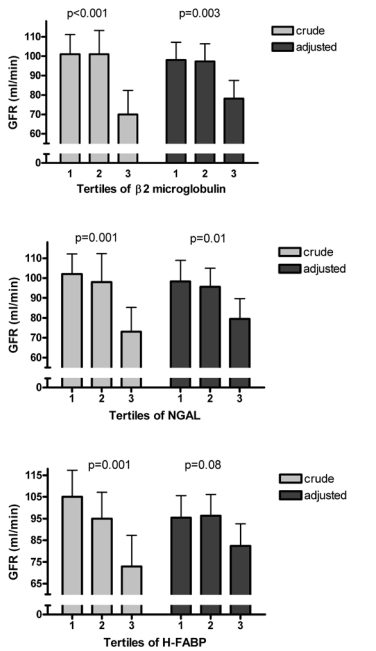


Figure 6. The associations between β2 microglobulin (upper panel), NGAL (middle panel) and H-FABP (lower panel), and ERBF are independent of albuminuria. Tertiles of the biomarkers are shown crude (light grey) and after adjustment of age, gender and albuminuria (dark grey).

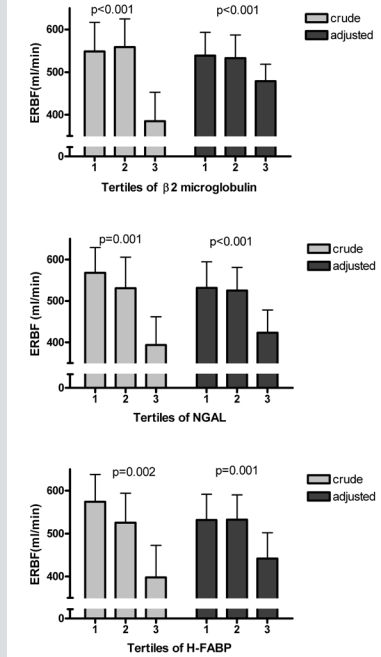


Figure 7. Associations between urinary biomarkers and Total Renal Volume.

Depicted are graphs of the urinary biomarkers under study that were significantly associated with TRV (table 3).

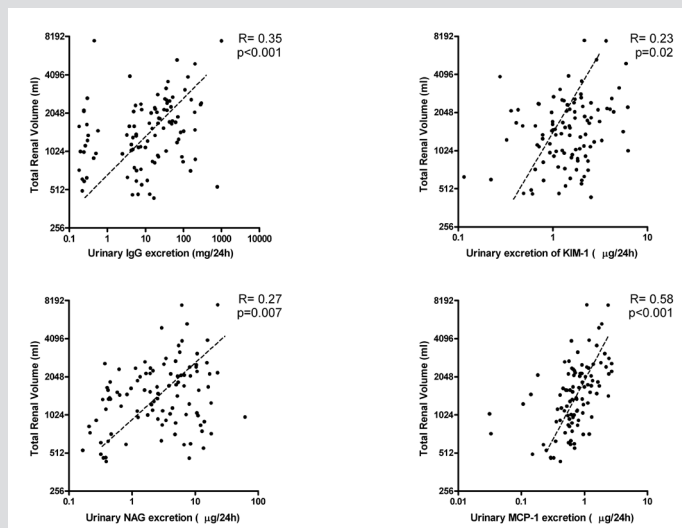


Table 6. Multivariable associations of various biomarkers with total renal volume after adjustment for age, gender (model 1) and additional adjustment for UAE (model 2).

	model	Urinary biomarker			UAE		
		β	95% CI for β	p-value	β	95% CI for β	p-value
UAE	1	-	-	-	0.21	0.14-0.29	<0.001
Glomerular							
IgG	1	0.10	0.05-0.14	0.001	-	-	-
	2	0.02	-0.04-0.08	0.5	0.19	0.09-0.29	<0.001
Proximal tubular							
KIM-1	1	0.23	0.09-0.38	0.002	-	-	-
	2	0.15	0.01-0.28	0.04	0.19	0.12-0.27	<0.001
B2MG	1	0.05	-0.02-0.13	0.15	-	-	-
	2	-0.01	-0.08-0.06	0.8	0.22	0.14-0.29	<0.001
NAG	1	0.09	0.01-0.18	0.04	-	-	-
	2	0.03	-0.05-0.11	0.5	0.20	0.13-0.28	<0.001
NGAL	1	0.22	0.12-0.32	<0.001	-	-	-
	2	0.12	0.02-0.23	0.03	0.17	0.09-0.25	<0.001
Distal tubular							
H-FABP	1	0.10	-0.02-0.23	0.11	-	-	-
	2	0.01	-0.11-0.12	0.9	0.23	0.15-0.31	<0.001
Inflammatory							
MIF	1	0.02	-0.13-0.17	0.8	-	-	-
	2	-0.07	-0.20-0.06	0.3	0.22	0.14-0.30	<0.001
MCP-1	1	0.40	0.27-0.52	<0.001	-	-	-
	2	0.28	0.14-0.42	<0.001	0.12	0.04-0.20	0.005

Beta's, confidence intervals and p-values were calculated using multivariable linear regression.
Dependent variable is log base 2 transformed TRV, independent variables are
the log base2 transformed 24h excretions of the various urinary biomarkers.
Model 1: adjusted for age and gender
Model 2: adjusted for age, gender and albuminuria.
Abbreviations are: IgG, immunoglobulin G; β2 MG, β2 microglobulin; KIM-1, Kidney Injury Molecule 1; NAG, N-acetyl-β-D-glucosaminidase; NGAL, Neutrophil Gelatinase-Associated Lipocalin; H-FABP, Heart-type Fatty Acid Binding Protein; MIF, macrophage migration inhibitory factor; MCP-1, monocyte chemotactic protein-1.

Sensitivity analyses

The associations between urinary biomarker excretions with functional and structural measures in ADPKD were essentially the same as the associations between urinary biomarker/creatinine ratios and these functional and structural measures. When all analyses were repeated including only ADPKD subjects with a mGFR >60 mL/min (n=79, not performed with mGFR as a reference test), the results of the crude associations (Table 3) remained the same, except for the association between H-FABP and ERBF, that now did not reach significance when only subjects with an mGFR > 60 mL/min were included.

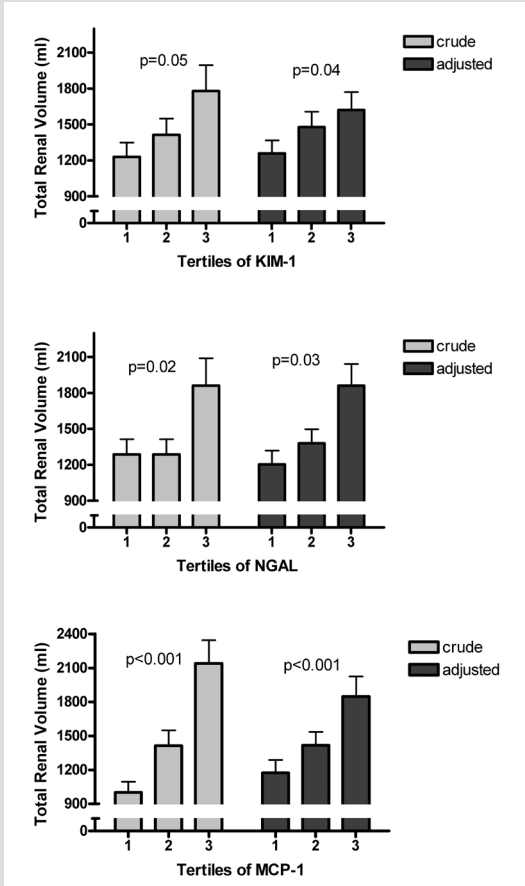


Figure 8. The associations between KIM-1 (upper panel), NGAL (middle panel), and MCP-1 (lower panel), and total renal volume are independent of albuminuria. Tertiles of the biomarkers are shown crude (light grey) and after adjustment of age, gender and albuminuria (dark grey).

DISCUSSION

The most important finding of this study is that all urinary biomarkers, be it for glomerular, proximal tubular, distal tubular damage or for inflammation, were elevated in ADPKD patients, when compared to healthy controls. Furthermore, NGAL was associated with renal blood flow and total renal volume, independent of albuminuria and is therefore an interesting candidate marker to predict disease progression. In addition to that, β2 microglobulin and H-FABP were inversely associated with glomerular filtration rate and effective renal blood flow, independent of albuminuria and KIM-1, NGAL and MCP-1 were positively associated with total renal volume, independent of albuminuria. Albuminuria correlated well with glomerular filtration rate, effective renal blood flow and total renal volume.

We found all urinary biomarkers to be elevated in ADPKD. According to our study, both glomerular, proximal- and distal tubular and the inflammatory marker MCP-1 were

elevated in ADPKD compared to healthy controls, suggesting the disease (indirectly) affects multiple parts of the nephron. Glomerular markers were more markedly elevated in the ADPKD patients than tubular damage markers. When patients are studied with a relatively well preserved eGFR (>60 ml/min per 1.73 m²), a relatively well preserved renal blood flow (>500 ml/min) or a relatively modest total renal volume (<1000 ml) all urinary biomarkers (except H-FABP and MIF) were also increased, indicating that the increase in urinary biomarkers occurs early in the disease process. That H-FABP excretion is elevated only later in the disease process is in contrast with literature that suggests that the main site of cystogenesis is distal in the tubules[39]. Of note, we found a striking correlation between IgG and albuminuria, which could suggest albuminuria in ADPKD is predominantly glomerular in origin. Furthermore, biomarkers were associated with either glomerular filtration rate and renal blood flow or total renal volume. Also in the ROC curves, the biomarkers with highest area under the curve are different for TRV and ERBF/mGFR. NGAL was the only urinary biomarker that remained associated with mGFR, effective renal blood flow and with total renal volume.

An increased urinary albumin excretion is a risk factor for ADPKD disease progression (renal growth and renal function deterioration).[9] Apart from albuminuria however, the studies that have been published on urinary biomarkers in ADPKD, are nearly always performed in small sample size populations and not corroborated by others. Urinary β2 microglobulin was elevated in 14 ADPKD patients compared to 6 healthy controls.[15] KIM-1 was expressed in murine polycystic kidneys, but not in normal kidneys.[40] To our knowledge however, urinary KIM-1 in human ADPKD has never been measured, nor are we aware of any studies describing urinary NAG levels in ADPKD. For NGAL, serum and urinary levels were higher in 26 ADPKD patients compared to 26 healthy volunteers.[16] Urinary fatty acid binding protein excretion is previously described to be elevated in ADPKD.[41] However, in this study liver-fatty acid binding protein was measured and not heart fatty acid binding protein, as in our study. Increased levels of MCP-1 in cyst mural cells and in urine was associated with monocyte accumulation within the renal interstitium in a rat model of ADPKD.[42] Also, in ADPKD patients, an increased urinary excretion of MCP-1 has been described.[17;18] MCP-1 is thought to play a critical role in the responses of macrophage migration inhibition factor (MIF). No studies so far have described urinary MIF excretion in ADPKD as yet. In one study, in polycystic kidney disease, multiple urinary biomarker tests were compared, namely albuminuria and urinary β-N-acetlyhexosaminidase and its isoenzymes.[43]

The question arises whether the markers we measured are only markers of disease activity, or whether they are also involved in the pathophysiologic process of cyst formation. Experimental studies suggest that KIM-1 is probably involved in phagocytosis.[44] It is also an endogenous ciliary protein[45] and could be involved in regulating flow-induced calcium signalling.[46] NGAL suppressed cyst growth by *PKD1* null cells in vitro and in mice.[47] Because of the observational nature of our study, we can only speculate whether the markers we found are indeed involved in the pathophysiology of ADPKD.

We acknowledge that our study has limitations. First, our study is cross-sectional, which could induce selection bias and makes that we are not able to look at associations between the markers and prediction of disease progression. Future studies will have to establish the clinical utility of these urinary biomarkers in predicting prognosis or response to therapy. Second, being a single centre study, our study needs of course external validation. Third, we do not know whether the found elevation of urinary biomarkers is specific for ADPKD or could also occur in non-ADPKD patients with chronic kidney disease. The increased urinary biomarker excretion could be caused by non-specific injury rather than the cystogenic process. Of note, we investigated whether urinary biomarkers can improve the assessment of disease severity in clinical practice. Therefore we did not take serum levels into account. Assessment of albuminuria is often performed in clinical practice, we attempted to identify urinary biomarkers that are of additional value to albuminuria. Strengths of our study are that we measured multiple markers in a relatively large cohort, consisting of 102 ADPKD patients, at different stages of the disease, and associated these biomarkers with gold standard measurements of renal blood flow and total renal volume, enabling a comparison between these biomarkers.

In summary, we found urinary biomarkers from all segments of the nephron to be elevated in ADPKD patients compared to healthy controls. NGAL was associated with both renal blood flow, as well as total with renal volume, independent of albuminuria. We found associations that were independent of albuminuria between β2 microglubulin and H-FABP versus effective renal blood flow and of KIM-1, NGAL and MCP-1 and total renal volume. Based on these cross-sectional data, we hypothesize that determination of β2 microglobulin, KIM-1, H-FABP, MCP-1, and especially NGAL could have additional value in clinical practice to assess disease severity in ADPKD.

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8B

URINARY BIOMARKERS IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE – IS THERE NO PROGNOSTIC VALUE?

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To the editor: We read with interest the article by Parikh et al, in which they showed that levels of neutrophil gelatinase-associated lipocalin (NGAL) and interleukin (IL-)18 did not correlate with changes in kidney volume or estimated glomerular filtration rate (eGFR) during follow-up in autosomal dominant polycystic kidney disease (ADPKD) patients relatively early in their disease [1]. Therefore, it was suggested that urinary biomarkers of kidney injury have no clinical prognostic utility.

Recently, we measured several biomarkers (glomerular, tubular, and inflammatory) in urine samples (stored for 0.7 years at -80°C) of 102 ADPKD patients and found in a cross-sectional study significant associations between several urinary biomarkers, including NGAL, and measures of ADPKD severity [2]. After having read the publication by Parikh et al, we investigated the association of these markers with decline in eGFR (chronic kidney disease epidemiology collaboration; CKD-EPI) in the patients of whom follow-up data were available without experimental intervention (N=46, age 40 ±14 years, 48% male, baseline eGFR 73 ±37 ml/min/1.73m^2, eGFR change during 2.6 y follow-up -2.6 ±3.2 ml/min/1.73m^2/y). We found significant inverse associations between baseline urinary biomarker excretion of albumin, IgG, KIM-1, and MCP-1 and change in eGFR during follow-up (Table 1). These associations remained significant after adjustment for age, gender, and baseline eGFR. Similar to Parikh et al, we found no significant association between NGAL and change in eGFR, neither crude (p=0.07) nor adjusted for age, sex and baseline eGFR (p=0.47). Urinary IL-18 was not measured.

Importantly, we have previously shown that frozen storage decreases the measured concentration of urinary biomarkers, such as NGAL, and induces more variability, especially after longer storage [3]. The decline or increased variability in marker concentration that is induced by frozen storage can therefore reduce the association that, in reality, is present. We have shown this latter phenomenon for urinary albumin concentration in non-ADPKD subjects [4].

Given these findings, we caution against a too skeptical view toward the utility of urinary biomarkers to predict disease progression in ADPKD. Our data indicate that some of these markers may be useful. More research investigating their prognostic value in ADPKD is definitely needed.

Table 1. Association between slope of eGFR (CKD-EPI) and log-transformed urinary biomarker excretion.

	Crude		Adjusted for age, gender and baseline eGFR	
	Std. B	p-value	Std. B	p-value
Albumin (mg/24hr)	-0.542	<0.001	-0.474	0.005
IgG (mg/24hr)	-0.419	0.004	-0.310	0.044
KIM-1 (µg/24hr)	-0.347	0.018	-0.447	0.001
MCP-1 (µg/24hr)	-0.483	0.001	-0.390	0.006

Abbreviations: CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; IgG, Imunoglobulin G; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemotactic protein 1.

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9

SHORT-TERM EFFECTS OF TOLVAPTAN IN SUBJECTS WITH ADPKD AT VARIOUS LEVELS OF KIDNEY FUNCTION

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Submitted

ABSTRACT

Tolvaptan, a vasopressin V2-receptor antagonist, delays disease progression in autosomal dominant polycystic kidney disease (ADPKD) in subjects with relatively preserved kidney function. In this study, we investigated the associations between kidney function and short-term response of tolvaptan treatment on efficacy variables (markers for aquaresis and kidney injury). Subjects with a wide range of kidney function (18-148 mL/min) were studied at baseline and after 3 weeks treatment with tolvaptan (up to 120 mg/day). GFR was assessed as ^{125}I-iothalamate clearance and total kidney volume (TKV) by MR imaging. Twenty seven subjects (52% male; 46 ±10 y, GFR 69 ±39 mL/min, TKV 2.15 (1.10 – 2.77) L) were included. At baseline, lower GFR was associated with higher TKV (p=0.002), 24hr urinary volume (p=0.05), and lower urinary osmolality (p<0.001). Three week treatment of tolvaptan induced an increase in urine volume, free water clearance and a decrease in urine osmolality, TKV and urinary excretion of damage markers for various nephron segments. Urine osmolality during tolvaptan treatment was low (mean 149 mOsm/kg), not associated with GFR (p=0.7). The change in 24hr urine osmolality and volume after 3 weeks of treatment was less in subjects with lower GFR (p=0.003; p<0.001, resp.). Baseline GFR was not associated with the absolute change in TKV (p=0.5), whereas the association of GFR with percentage decrease in TKV reached borderline significance (p=0.06). Importantly, on treatment absolute as well as change in fractional free water clearance was higher in subjects with lower kidney function (p<0.001; p=0.001, resp.), suggesting that subjects with impaired GFR had more response per functioning nephron. In conclusion, response to tolvaptan regarding urinary volume, osmolality and TKV is lower in subjects with impaired kidney function when using absolute values, but are similar when using relative changes. Based on our results, we hypothesize that effects on tolvaptan are not lower in subjects with lower GFR due to decreased sensitivity for tolvaptan, but could be due to less functioning renal mass in these subjects.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary kidney disease which leads to cyst formation in especially the kidneys, resulting in kidney enlargement and function loss. Fifty percent of affected subjects need renal replacement therapy in their sixth decade of life [1].

Experimental studies suggested that arginine vasopressin (AVP) may have a central role in the pathophysiology of this disease. Human studies showed that in ADPKD patients, higher AVP levels are associated with a decrease in kidney function and an increase in total kidney volume (TKV) during follow-up [2]. Blocking the AVP V2-receptors is therefore a promising therapeutic intervention in this disease. Several experimental studies showed that AVP V2-receptor antagonists slow the rate of cyst development and kidney growth in various models for cystic kidney disease [3–7]. In 20 ADPKD subjects the AVP V2-receptor antagonist tolvaptan given at low dose (45/15 mg split dose) caused a decrease in TKV after 1 week of treatment[8]. A study by Highashihara et al[9] suggested that long-term use of this drug is associated with less increase in TKV and less decrease in kidney function when compared to historical ADPKD control subjects that were matched for several patient characteristics. Recently, the TEMPO 3:4 Study [10] prospectively showed that use of tolvaptan, administered in doses between 45/15 and 90/30 mg/day as a split dose, slowed the increase in TKV and the decline in kidney function over a 3-year period in 1445 patients with ADPKD [11]. All three aforementioned studies were performed in ADPKD subjects with relatively preserved kidney function. Animal as well as human studies have suggested that the efficacy of AVP receptor antagonists may be lower when given at later stage disease. [6,8]

The aim of the present study was to determine whether the renal hemodynamic effects and phamacodynamic efficacy of tolvaptan in ADPKD subjects is dependent on kidney function. For that reason we investigated short-term responses on various efficacy parameters to target therapeutic doses of this drug in ADPKD subjects with a wide range of kidney function, including subjects with glomerular filtration rate (GFR) <30 mL/min/1.73m^2, and whether therapy induced changes in these parameters are dependent on baseline kidney function. The renal hemodynamic results of this study showed that change in GFR, effective renal plasma flow and filtration fraction were not different between subjects with lower GFR kidney function compared with higher GFR. [12] Pharmacodynamic efficacy variables were also assessed to include parameters indicative for the effects of this drug on aquaresis (copeptin concentration, urine volume, urine osmolality and free water clearance) and kidney injury (total kidney volume and urinary biomarker excretion).

Table 1. Baseline characteristics

N	27
Male (%)	52
Age (years)	46.3 ± 9.8
BMI (kg/m²)	25.7 ± 4.1
Mean arterial pressure (mmHg)	89 (86; 96)
Antihypertensive drugs (yes, %)	88.9
ACEi/ARB (yes,%)	85.2
Serum creatinine (umol/L)	135 (77; 263)
Plasma copeptin (pmol/L)	9.6 (4.8; 25.5)
Plasma osmolality (mOsm/kg)	286 (281; 293)
24 hour urine	
- volume (mL/24hr)	2584 ± 839
- osmolality (mOsm/kg)	359 (289; 425)
- albumin (mg/24hr)	45 (20; 133)
- IgG (mg/24 hr)	9.1 (3.7; 21.9)
- NGAL (ug/24hr)	72.8 (44.7; 200.7)
- H-FABP (ug/24hr)	1.8 (0.6; 5.9)
- MCP-1 (ug/24hr)	0.9 (0.7; 1.4)
- KIM-1 (ug/24hr)	1.6 (1.0; 2.0)
Free water clearance (L/24 hr)	-0.6 (-1.2; 0.1)
Fractional free water clearance (%)	-0.5 (-1.0; 0.1)
mGFR (mL/min)	69.1 ± 38.6
TKV (L)	2.15 (1.10; 2.77)

Means ±SD and Medians (IQR). Plasma osmolality was measured before tracer infusion started Mean arterial pressure (calculated with the mean of three blood pressure measurements after 10 min rest) was measured 10 min after the start of tracer infusion. Abbreviations: BMI, body mass index; ACEi, ACE inhibition; ARB, angiotensin II receptor blockers; IgG, immunoglobulin G; NGAL, neutrophil gelatinase-associated lipocalin; H-FABP, heart-type fatty acid binding protein; MCP-1, monocyte chemotactic protein 1; KIM-1, kidney injury molecule 1; mGFR, measured glomerular filtration rate; TKV, total kidney volume.

RESULTS

Twenty nine subjects were included, of which twenty seven subjects completed the study (n=9 per eGFR stratum); 2 subjects withdrew because of adverse events (one after 3 days of treatment because of polyuria and another after 13 days because of xerostomia). The characteristics of the 27 subjects who completed the study are given in Table 1. All subjects completed the study on the 90/30 mg tolvaptan per day split-dose regimen, except one subject who did not tolerate the highest dose and completed the study on 60/30 mg. Mean GFR was 69.1 ±38.6 mL/min, with a wide range (18-148 mL/min).

At baseline, GFR was significantly negatively associated with TKV, plasma osmolality, plasma copeptin, 24 hour urine volume, free water clearance and fractional free water clearance and significantly positively associated with urine osmolality (Table 2). This indicates that subjects with lower GFR had larger kidneys, and higher plasma osmolality,

Table 2. Associations between baseline GFR and study variables at baseline (left) and during tolvaptan treatment (right).

	Baseline		Treatment	
	Beta	P-value	Beta	P-value
Plasma				
- copeptin (ln)	-0.764	<0.001	-0.632	0.001
- osmolality	-0.700	<0.001	-0.739	<0.001
C_{max}	NA	NA	-0.025	0.901
24 hour urine				
- volume	-0.374	0.054	0.380	0.051
- osmolality (ln)	0.654	<0.001	0.073	0.719
- albumin (ln)	-0.557	0.004	-0.561	0.004
- IgG	-0.507	0.010	-0.671	<0.001
- NGAL (ln)	-0.649	<0.001	-0.726	<0.001
- HFABP (ln)	-0.540	0.005	-0.647	<0.001
- MCP-1 (ln)	0.082	0.697	-0.461	0.020
- KIM-1	0.068	0.748	-0.119	0.572
FWC	-0.668	<0.001	0.228	0.252
Frac FWC	-0.415	0.031	-0.757	<0.001
TKV (ln)	-0.566	0.002	-0.574	0.002

Abbreviations: IgG, immunoglobulin G; NGAL, neutrophil gelatinase-associated lipocalin; HFABP, heart-type fatty acid binding protein; MCP-1, monocyte chemotactic protein 1; KIM-1, kidney injury molecule 1; TKV, total kidney volume; FWC, free water clearance; frac FWC, fractional FWC; C_{max}, maximum tolvaptan concentration; NA, not applicable

plasma copeptin level, 24 hr urine volume and free water clearance, and lower urine osmolality. We also found significant associations between GFR and the excretion of several urinary biomarkers. Lower GFR was associated with higher urinary excretions of albumin, IgG, NGAL and HFABP, whereas we found no association between GFR and urinary excretion of KIM-1 and MCP-1.

Table 2 also shows the associations between baseline GFR and the aforementioned variables measured during tolvaptan use. Whereas in general results were similar, three exceptions were noted. The associations of baseline GFR with urine osmolality and free water clearance lost significance, and the association with 24 hour urine volume reversed; i.e. subjects with lower GFR had lower 24hr urine volumes during tolvaptan use.

The effects after 3 weeks treatment with tolvaptan are given in Table 3. A significant decrease in TKV and GFR was observed, as well as in all other measured variables. This decrease was not seen in urinary MCP-1 and KIM-1 concentration, which remained essentially unchanged. Plasma osmolality, copeptin concentration and urine volume, in contrast, increased significantly. All of these changes were reversible after 3 weeks withdrawal of tolvaptan, only TKV remained slightly, but significantly lower than the baseline value (median volume 2103 (1080 – 2737) mL; change -1.7 ±2.9%, p=0.006).

Some of the changes in study variables during tolvaptan treatment were associated with baseline GFR (Table 4). For instance, the lower GFR, the less increase in urine volume, and the less decrease in urine osmolality and GFR was found. A lower GFR was

Table 3. Changes in study variables during tolvaptan treatment

	Baseline	Final treatment	Absolute change	Percentage change	P-value
Plasma osmolality (mOsm/kg)	287.4 ± 7.0	290.6 ± 7.5	3.1 ± 3.1	1.1 ± 1.1	<0.001
Plasma copeptin (pmol/L)	9.6 (4.8; 25.5)	28.4 (16.6; 41.1)	15.8 (11.4; 20.7)	182 (109; 288)	<0.001
Urine volume (mL/24hr)	2584 ± 839	5930 ± 1790	3347 ± 1689	146.0 ± 99.9	<0.001
Urine osmolality (mOsm/kg)	359 (289; 425)	139 (126; 173)	-208 (-254; -136)	-55 ± 17	<0.001
Albuminuria (mg/24hr)	45 (20; 133)	43 (21; 105)	-7 (-13; 8)	-10 ± 39	0.029
IgG (mg/24 hr)	9.1 (3.7; 21.9)	1.3 (0.1; 12.4)	-4 (-9; -1)	-74 (-98; -18)	0.001
NGAL (ug/24hr)	72.8 (44.7; 200.7)	56.0 (24.8; 167.5)	-10 (-20; 4)	-21 ± 33	0.022
HFABP (ug/24hr)	1.8 (0.6; 5.9)	0.5 (0.2; 4.4)	-0.8 ± 3.0	-57 (-79; 14)	0.009
MCP-1 (ug/24hr)	0.9 (0.7; 1.4)	0.8 (0.6; 1.3)	-0.2 (-0.5; 0.3)	-15 (-73; 19)	0.774
KIM-1 (ug/24hr)	1.5 ± 0.8	1.6 ± 0.8	0.0 ± 0.7	10 ± 52	0.856
FWC (L/24hr)	-0.5 ± 1.0	3.0 ± 1.3	3.5 ± 1.7		<0.001
Fractional FWC (%)	-0.2 ± 1.0	4.2 ± 2.4	4.4 ± 2.3		<0.001
GFR (mL/min)	69.1 ± 38.6	64.4 ± 35.7	-3.0 (-9.0; -0.3)	-5.4 ±8.0	0.002
TKV (mL)	2147 (1100; 2767)	2052 (1040; 2690)	-60 (-103; -16)	-3.7 ± 3.0	<0.001

Means ±SD and Medians (IQR). Abbreviations: IgG, immunoglobulin G; NGAL, neutrophil gelatinase-associated lipocalin; HFABP, heart-type fatty acid binding protein; MCP-1, monocyte chemotactic protein 1; KIM-1, kidney injury molecule 1; FWC, free water clearance

furthermore associated with less decrease in TKV when expressed as percentage, but not when expressed as absolute change (Figure 1). The same held true for change in copeptin. Free water clearance increased also less in subjects with lower GFR, but when free water clearance is expressed normalized for GFR (fractional FWC) it shows that subjects with lower GFR had a more distinct increase (Figure 2). The change in excretions of urinary biomarkers during tolvaptan use was not associated with baseline GFR, except for IgG and MCP-1.

Subjects collected 24 hour urines in three portions. With tolvaptan treatment, urine volumes increased and osmolality decreased in all three portions (Table 5). Changes in urine osmolality during day and evening time were significantly higher than change in urine osmolality during night time. Change in urine volume was significantly higher during evening time compared with day and night time. During tolvaptan treatment osmolality of all urine samples was lower than plasma osmolality in all subjects.

Of note, there was no significant association between baseline GFR and maximal tolvaptan concentration (C_{max}, mean 745 ±305 ng/mL, p=0.73), nor area under the concentration curve ($AUC_{0\text{-}5h}$, mean 2673 ±956 h*ng/ml, p=0.48). In general C_{max} and AUC_t were not associated with absolute as well as percentage changes in the study variables listed in Supplementary Table 1. Similarly, baseline and on treatment copeptin concentration were not associated with changes in these study variables, except for absolute change in fractional free water clearance. The higher the copeptin level at baseline, the more increase in fractional free water clearance was found (Supplementary Table 2).

Table 4. Associations between baseline GFR and percentage and absolute change in study variables during tolvaptan treatment.

	Beta	P-value
Plasma copeptin (ln)		
- percentage change	0.611	0.001
- absolute change	-0.305	0.138
Plasma osmolality		
- percentage change	-0.196	0.327
- absolute change	-0.209	0.296
Urine volume		
- percentage change	0.633	<0.001
- absolute change	0.588	0.001
Urine osmolality		
- percentage change	-0.546	0.003
- absolute change	-0.716	<0.001
Albuminuria		
- percentage change	-0.190	0.363
- absolute change	-0.064	0.761
IgG		
- percentage change (ln)	-0.684	<0.001
- absolute change	-0.202	0.333
NGAL		
- percentage change	-0.386	0.057
- absolute change	-0.373	0.066
HFABP		
- percentage change (ln)	-0.261	0.208
- absolute change	0.120	0.567
MCP-1		
- percentage change	-0.467	0.019
- absolute change (ln)	-0.387	0.056
KIM-1		
- percentage change	-0.047	0.823
- absolute change	-0.181	0.385
FWC		
- absolute change	0.566	0.002
fractional FWC		
- absolute change	-0.584	0.001
GFR		
- percentage change	-0.336	0.087
- absolute change	-0.500	0.008
TKV		
- percentage change	-0.361	0.064
- absolute change	-0.053	0.795

Spearman correlation coefficient is given for absolute change in urine osmolality, albuminuria, TKV, GFR, IgG, NGAL and for percentage change in MCP. For all other associations Pearson correlation coefficient is shown.

Table 5. Urine osmolality and volume collected during daytime (7.00 am – 5.00 pm), evening time (5.00 pm – bedtime) and night time (bedtime – 7.00 am) at baseline and during tolvaptan treatment.

	Baseline	During treatment	Change (%)	P-value
Daytime				
- Urine osmolality (mOsm/kg)	363 (283; 478)	146 (118; 171)	-57 (-71; -50)	<0.001
- Urine volume (mL)	1020 (820; 1410)	2350 (1720; 2880)	106 (40; 185)	<0.001
Evening				
- Urine osmolality (mOsm/kg)	341 (273; 532)	136 (110; 150)	-60 (-73; -48)	<0.001
- Urine volume mL)	600 (440; 820)	2000 (1360; 2600)	177 # (129; 320)	<0.001
Nighttime				
- Urine osmolality (mOsm/kg)	327 (273; 381)	154 (134; 188)	-51* (-62; -38)	<0.001
- Urine volume (mL)	670 (500; 1140)	1640 (1230; 2150)	87 (44; 192)	<0.001

Medians (IQR). Differences were tested with the paired Wilcoxon signed ranks test.
* Significant versus change during daytime (p=0.012) and evening time (p<0.001).
# Significant versus change during daytime (p=0.005) and night (p=0.002).

Figure 1. Association between baseline GFR and absolute (figure a, b=-0.053, p=0.795) and percentage (figure b, b=-0.361, p=0.064) change in total kidney volume during tolvaptan treatment.

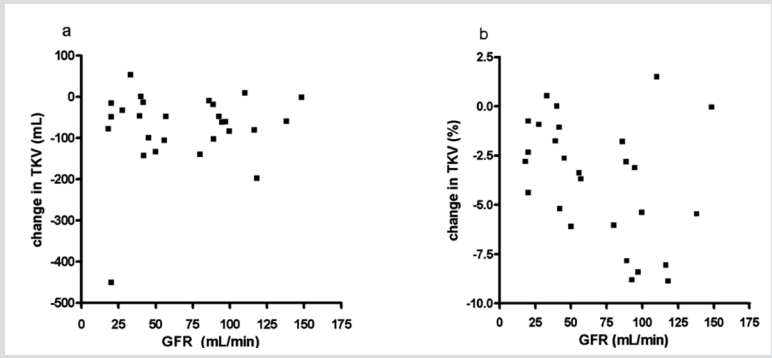
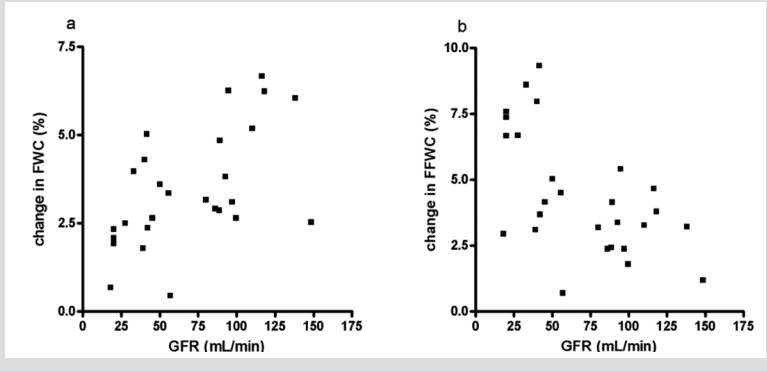


Figure 2. Association between baseline GFR and absolute change in FWC (figure a, b=0.566, p=0.002) and FFWC (figure b, b=-0.584, p=0.001) during tolvaptan treatment.



Supplementary Table 1. Association Cmax and AUCt with percentage and absolute change in efficacy variables.

	Cmax		AUCt	
Change in	R	P-value	R	P-value
Plasma osmolality				
- percentage change	0.273	0.168	0.202	0.312
- absolute change	0.283	0.152	0.215	0.281
Plasma copeptin				
- percentage change (ln)	-0.065	0.756	-0.206	0.323
- absolute change (ln)	-0.026	0.902	0.021	0.922
Urine volume				
- percentage change	0.001	0.994	-0.046	0.821
- absolute change	0.008	0.969	-0.021	0.918
Urine osmolality				
- percentage change	-0.049	0.806	-0.039	0.846
- absolute change	0.016	0.935	0.013	0.949
Albuminuria				
- percentage change	-0.119	0.572	0.015	0.943
- absolute change	-0.224	0.282	-0.117	0.579
IgG				
- percentage change (ln)	-0.097	0.643	-0.006	0.978
- absolute change	-0.188	0.367	-0.132	0.531
NGAL				
- percentage change	-0.299	0.146	-0.221	0.287
- absolute change	-0.359	0.078	-0.284	0.169
HFABP				
- percentage change (ln)	-0.170	0.417	-0.227	0.275
- absolute change	-0.238	0.252	-0.283	0.171
MCP-1				
- percentage change	0.055	0.795	0.082	0.697
- absolute change (ln)	0.109	0.605	0.143	0.496
KIM-1				
- percentage change	0.230	0.269	0.097	0.644
- absolute change	0.024	0.909	-0.041	0.844
TKV				
- percentage change	0.005	0.980	0.125	0.533
- absolute change	-0.141	0.483	-0.072	0.722
GFR				
- percentage change	0.230	0.248	0.217	0.277
- absolute change	0.230	0.249	0.274	0.167
FWC				
- absolute change	0.023	0.910	0.002	0.991
fractional FWC				
- absolute change	0.207	0.300	0.297	0.132

Pearson. Percentage change and absolute change in copeptin were ln-transformed before analysis. For absolute change (deltas) in urine osmolality, albuminuria, TKV, GFR, IgG, NGAL, and for percentage change in MCP, Spearman correlation is given.

Abbreviations: IgG, immunoglobulin G; NGAL, neutrophil gelatinase-associated lipocalin; HFABP, heart-type fatty acid binding protein; MCP-1, monocyte chemotactic protein 1; KIM-1, kidney injury molecule 1; TKV, total kidney volume; GFR, glomerular filtration rate; FWC, free water clearance.

Supplementary Table 2. Association baseline (n=27) and on treatment (N=25) plasma copeptin concentration and percentage and absolute change in efficacy variables.

	Ln Copeptin baseline		Ln Copeptin on treatment	
Change in	R	P-value	R	P-value
Plasma osmolality				
- percentage change	0.003	0.990	0.053	0.801
- absolute change	0.022	0.914	0.068	0.747
Plasma copeptin				
- percentage change (ln)	-0.804	<0.001	-0.448	0.025
- absolute change (ln)	0.455	0.022	0.794	<0.001
Urine volume				
- percentage change	-0.261	0.188	-0.016	0.938
- absolute change	-0.258	0.195	-0.037	0.862
Urine osmolality				
- percentage change	0.018	0.930	-0.155	0.460
- absolute change	0.285	0.149	0.191	0.361
Albuminuria				
- percentage change	0.363	0.075	0.330	0.116
- absolute change	0.066	0.754	0.054	0.804
IgG				
- percentage change (ln)	0.425	0.034	0.295	0.162
- absolute change	0.146	0.486	0.189	0.377
NGAL				
- percentage change	0.348	0.088	0.386	0.063
- absolute change	0.255	0.218	0.309	0.142
HFABP				
- percentage change (ln)	0.168	0.423	0.288	0.173
- absolute change	0.037	0.860	0.045	0.836
MCP-1				
- percentage change	0.354	0.083	0.163	0.445
- absolute change (ln)	0.372	0.067	0.259	0.222
KIM-1				
- percentage change	0.131	0.533	0.049	0.819
- absolute change	0.278	0.179	0.069	0.748
TKV				
- percentage change	0.316	0.108	0.152	0.468
- absolute change	-0.069	0.732	-0.092	0.661
GFR				
- percentage change	0.179	0.371	0.031	0.882
- absolute change	0.317	0.107	0.118	0.574
FWC				
- absolute change	-0.143	0.477	0.012	0.954
fractional FWC				
- absolute change	0.833	<0.001	0.761	<0.001

Pearson correlation. Percentage change and absolute change in copeptin were ln-transformed before analysis. For absolute change (deltas) in urine osmolality, albuminuria, TKV, GFR, IgG, NGAL, and for percentage change in MCP, Spearman correlation is given.
Abbreviations: IgG, immunoglobulin G; NGAL, neutrophil gelatinase-associated lipocalin; HFABP, heart-type fatty acid binding protein; MCP-1, monocyte chemotactic protein 1; KIM-1, kidney injury molecule 1; TKV, total kidney volume; GFR, glomerular filtration rate; FWC, free water clearance.

DISCUSSION

In this study we found that 3 week treatment with tolvaptan caused an increase in urine volume, free water clearance and plasma copeptin and a decrease in urine osmolality. Furthermore, tolvaptan induced a decrease in total kidney volume and urinary excretion of biomarkers that are associated with damage to various nephron segments. The changes in urine volume, free water clearance, urine osmolality and TKV were less pronounced in subjects with lower baseline GFR when using absolute values. However, when using relative changes, the effects were similar in subjects with lower GFR compared with higher GFR. In these subjects with lower GFR, however, the increase in fractional free water clearance was most distinct.

Besides these findings, we found a significant association between GFR at baseline and markers for proximal (NGAL) and distal tubular damage (HFABP), but also with albuminuria (marker for glomerular and tubular damage) and IgG (marker for glomerular damage). These data corroborate previous findings in subjects with ADPKD [13] and indicate that this disease is associated not only with tubular damage, the nephron segment where cysts are formed, but also with glomerular damage. More importantly, this study is the first to investigate the effects of tolvaptan on these damage markers in ADPKD. We found after three weeks of treatment a significant decrease in urinary excretion of all biomarkers that were associated with GFR at baseline, suggesting that tolvaptan reduces tubular as well as glomerular kidney damage in subjects with ADPKD.

Experimental research suggested that in a more advanced stage of ADPKD V2 receptor antagonists may be less effective [6]. One of the possible reasons for this suggestion is the fact that in these subjects AVP is up regulated to compensate for the impaired urinary concentrating capacity in later stage disease. This led to the hypothesis that when the agonist (AVP) is increased, the dose of the antagonist (tolvaptan) should be increased [6]. Our data, however, show that higher plasma levels of tolvaptan did not result in more effects, and that there was no association between baseline (nor on treatment) copeptin level and most effects of tolvaptan.

In human ADPKD, Irazabal et al[8], based on the results of their study in which low dose tolvaptan (45/15 mg split dose regimen) was administered during one week, suggested also that tolvaptan may be less effective in lowering TKV in later stage ADPKD. We similarly observed that in subjects with lower GFR tolvaptan tended to have less effect on TKV when expressed as percentage change. Moreover, these subjects also had less increase in urinary volume and less decrease in urinary osmolality. These results can at first sight be interpreted as subjects with lower GFR showing less responsiveness to tolvaptan.

However, the present data show that there are also several indications that tolvaptan might have similar efficacy in ADPKD subjects with lower GFR. First, the absolute change in TKV with treatment was not associated with baseline GFR. It may well be that it is not possible to achieve more than the 60 mL decrease in kidney volume that we observed

during the limited time period of 3 weeks treatment with tolvaptan. Since subjects with lower GFR have higher TKV at baseline, this similar absolute change results in a percentage less change in TKV in subjects with low GFR at baseline, but that does not automatically indicate less efficacy of the drug. Second, and in analogy, subjects with lower GFR showed less decrease in urine osmolality during treatment. It should be taken into account, however, that their urine osmolality was already lower at baseline, probably because of the fact that subjects with lower GFR have less urine concentration capacity. The urine osmolality that was reached during treatment (mean 149 ± 35 mOsm/kg) was not dependent on baseline GFR (p=0.52). In fact in all subjects, urine osmolality was lower than plasma osmolality during treatment, in the 24hr urine samples, as well as in the day-, evening- and nighttime samples. This suggests that the AVP V2-receptor was adequately blocked during 24 hours in all patients, i.e. independent of GFR. Third, during use of tolvaptan, urinary excretion of all biomarkers indicating damage to various nephron segments decreased (except KIM-1). For nearly all markers absolute and percentage reductions were independent of GFR, suggesting that tolvaptan reduced damage to the various nephron segments also in subjects with lower GFR. Fourth, and most important, to reliably assess the renal physiological effects of tolvaptan it may be advisable to adjust for functioning nephron mass. This is done by studying free water clearance normalized for GFR, i.e. fractional free water clearance. The data with respect to this latter variable suggest that per functioning nephron most effect was observed in subjects with low baseline GFR.

Taking all the aforementioned points into considerations we hypothesize that ADPKD subjects with lower GFR might also benefit from long-term treatment with tolvaptan, as has been observed for subjects with relatively preserved GFR [11]. These data should be considered hypothesis generating, and that the renoprotective effect of tolvaptan in ADPKD subjects with impaired GFR can only be proven in long-term, large scale, randomized controlled trials.

We acknowledge that this study has limitations. First, the number of participating subjects is relatively small, which increases the chance of type I errors. It should be acknowledged that it is difficult to include large numbers of subjects in an intensive study as the present one. Furthermore, we found significant associations between baseline GFR and changes in most study variables, suggesting that the number of included subjects is sufficient to reach conclusions. Second, subjects took tolvaptan for only three weeks. Assessing long-term effects of this drug needs additional study. Strengths of this study are the inclusion of subjects with a wide range of GFR, and the fact that we measured GFR with a gold standard method, at three time points, instead of using estimated GFR. In addition, we measured kidney volume with the reference method (MR imaging) at these three time points, and subjects collected 24 hour urine samples in three portions (during day, evening and night time) providing good insight into the changes in urine osmolality and volumes.

In conclusion, response to tolvaptan on urinary volume, urinary osmolality and TKV is lower in ADPKD subjects with impaired kidney function when using absolute values. In contrast, in subjects with lower kidney function, the effect of tolvaptan on fractional free water clearance is more distinct. Based on these latter results, we hypothesize that the lower absolute response on urinary volume, urinary osmolality and TKV in subjects with impaired kidney function is not due to decreased sensitivity for tolvaptan, but could be due to less functioning renal mass or structural renal abnormalities. In addition, we found that absolute change in TKV, plasma copeptin and urinary biomarker excretions were not associated with baseline GFR, suggesting that ADPKD subjects with lower GFR might also benefit from long-term treatment with tolvaptan, as has been observed for subjects with relatively preserved GFR.

METHODS

Study population

Study participants were eligible when diagnosed with ADPKD based on the Ravine criteria [14] and had an age between 18 and 70 years. They were included by eGFR (MDRD equation [15]) in three strata (>60, 30-60 and <30 mL/min/1.73m^2) to ensure that inclusion was balanced to cover a wide range of kidney function.

Main exclusion criteria were: body mass index >35 kg/m^2, use of diuretics, pregnancy or breast-feeding, previous exposure to tolvaptan, risk factors for renal impairment other than ADPKD, recent renal surgery, diabetes mellitus, contraindications to MRI, critical electrolyte imbalances and uncontrolled hypertension. Participants with hypertension were treated with angiotensin I converting enzyme inhibitor or an angiotensin II receptor blocker, with addition of any other antihypertensive drug if needed (except diuretics).

This study was approved by the Ethical Board at the University Medical Center Groningen and performed in adherence to the ICH-GCP. Written informed consent was obtained from all subjects. The trial is listed on clinicaltrials.gov [NCT01336972]

Study design

Subjects were screened 2 – 42 days before dosing tolvaptan. Subjects were instructed to collect urine for 24 hours before every kidney function measurement test and not to drink alcohol or use any food or beverages containing methyl xanthines within 24 hours of kidney function testing to avoid effects on AVP signaling. Since tolvaptan is a weak CYP3A4 substrate, subjects were instructed not to use grapefruit or Seville oranges within 72 hours prior to dosing of tolvaptan.

One day before starting tolvaptan, subjects visited the clinic for kidney function and TKV measurements (baseline visit). The day after the baseline visit, subjects started

tolvaptan in a split-dose regimen with 45 mg in the morning and 15 mg approximately 8 hours after the first dose. After one week of treatment, in case this low dose was tolerated, subjects started an intermediate dose (60/30 mg/day split-dose), which after another week, if tolerated, was up-titrated to a split-dose regimen with 90/30 mg/day. On the last day of this 3 week treatment period, as well as 3 weeks after the last dose of tolvaptan, kidney function and volume were again measured. On the last day of treatment, the highest tolerated dose of tolvaptan was administered 30 minutes after the start of kidney function tracer infusion.

Because of the large number of variables measured in this intensive study protocol, the present manuscript focuses on effects of tolvaptan on efficacy variables, whereas the effects on renal hemodynamics, adverse events and safety are reported in detail elsewhere [12].

Measurements and calculations

On kidney function and kidney volume measurement days, subjects visited our clinic at around 7:45 a.m., while they were at least 4 hours fasting (but drinking water ad libitum). Blood samples were drawn at around 8:00 a.m., in which creatinine (Roche enzymatic assay), plasma and urine osmolality (freezing point depression) and copeptin were measured. Copeptin is a surrogate for AVP and was measured using a Chemiluminescence Immunoassay (CT-proAVP LIA; Thermofisher Scientific, Hennigsdorf, Germany) as described previously [16]. Free water clearance was calculated as urine flow minus the osmolar clearance. Osmolar clearance was calculated by the formula (urine osmolality * urine volume)/ plasma osmolality. Fractional free water clearance was calculated by dividing the free water clearance by the GFR.

Kidney function measurements used the constant infusion method of ^{125}I-iothalamate and ^{131}I-hippuran [17–19]. After drawing a timepoint-0 blood sample at around 8:00 a.m., a priming solution containing 20 mL of infusion solution (0.04 MBq of ^{125}I-iothalamate and 0.03 MBq of ^{131}I-hippuran) was given, followed by a constant infusion of 6 to 12 mL/h, with the lowest infusion rates in subjects with impaired kidney function on the basis of the serum creatinine at screening. Plasma concentrations of both tracers were allowed to stabilize during 1.5 hours which was followed by two 2-hour periods for simultaneous assessment of clearances of ^{125}I-iothalamate and ^{131}I-hippuran. Clearances were calculated as (U x V) / P_{iot} and (I x V) / P_{hipp}, respectively. Because urinary clearance of ^{131}I-hippuran equals plasma clearance in case of perfect urine collection, we routinely use the ratio of plasma-to-urinary clearance of ^{131}I-hippuran to correct urinary clearance of ^{125}I-iothalamate as measure of glomerular filtration rate (GFR) for voiding errors. The mean of the two GFR values is used for analyses.

Immediately after completing the kidney function test, subjects underwent a standardized abdominal MRI protocol without the use of intravenous contrast to measure TKV. Scanning was performed on a 3-T research MR scanner (Intera, Philips, Eindhoven,

the Netherlands) or a 1.5-T MR scanner (Magnetom Avanto, Siemens, Erlangen, Germany) in case of contraindications for the 3-T scanner which are not an issue on a 1.5-T MR. Cardio matrix coils were used for the 3-T scanner and body matrix and spine matrix coils were used for the 1.5 T MRI. Slice thickness was 4.0 mm. Alice™ software (Perceptive Informatics) is used to measure total kidney volume by calculating the volume of serial renal outlines which have been verified by independent radiologists familiar with ADPKD.

Plasma samples for measurement of tolvaptan concentration were collected at the final treatment visit (highest dose of tolvaptan) at six time points: 8.00 am (before the start of kidney function measurement), at 9.30 am (one hour after taking 90 mg of tolvaptan), 10.30 am; 11.30 am; 12.30 pm and 1.30 pm. Tolvaptan concentration was measured in these samples using a reverse-phase HPLC system with tandem mass spectrophotometric detection, as described previously [20]. The lower limit of quantitation was 5.00 ng/mL.

Samples from the 24 hour urine collections were used to measure the concentrations of urinary markers representing damage to different nephron segments. As glomerular damage marker we measured Immunoglobulin G (IgG) and albumin, as proximal tubular damage markers Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Kidney Injury Molecule 1 (KIM-1) [21], as distal tubular damage marker Heart-type Fatty Acid Binding Protein (H-FABP) [22] and as inflammatory marker monocyte chemotactic protein-1 (MCP-1) [23]. Urine was stored at –80°C and thawed before measurement. All biomarkers were measured by ELISA. For KIM-1, MCP-1 and NGAL, antibodies were obtained from R&D Systems (Minneapolis, MN). H-FABP and IgG antibodies were obtained from Hytest (Turku, Finland). If the measured value was below the lower limit of detection, we used the lower limit of detection value divided by 2. To calculate 24 hour excretions, concentrations were multiplied by 24 hour urine volume. In case subjects experienced macroscopic haematuria (possibly due to cyst rupture), they were excluded for the analysis of urinary biomarkers, because blood contamination may interfere with the assays measuring these biomarkers (N=2).

Subjects collected the aforementioned 24 hour urine in three parts: during daytime (7.00 am – 5.00 pm), evening (5.00 pm – bedtime) and nighttime (bedtime – 7.00 am). Urine osmolality and volume of these portions was measured at baseline and after treatment to investigate whether AVP V2-receptor blockade was effective during 24 hours.

STATISTICAL ANALYSES

Analyses were performed at the study center with SPSS version 20.0 (SPSS Inc., Chicago, IL). Parametric distributed variables are given as mean with standard deviation, whereas non-parametric distributed variables are given as medians with interquartile ranges. For all analyses a two-sided P less than 0.05 was considered to indicate statistical significance.

For Pearson correlations tests all variables with a skewed distribution were logarithmically transformed to fulfil the requirement of normal distribution of the residuals. Pearson, or Spearman in case a variable had no normal distribution even after log-transformation, correlation test was performed to assess associations between baseline GFR and (changes in) various variables. Differences between baseline and final treatment variables were tested with a paired T-test or, in case of non-parametric distributed variables, with a Wilcoxon signed ranks test.

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DISCLOSURES

FSC, HBK, DO and JO are employees of Otsuka. RTG is member of the steering committee for the Otsuka TEMPO 3:4 trial. JS was an employee of ThermoFisher Scientific, B.R.A.H.M.S. Biomarkers, the company that manufactures and holds patent rights on the copeptin assay. GEE and WvO are employees of Haemoscan. The other authors declared no competing interests. This study was funded by Otsuka Pharmaceutical Development & Commercialization, Inc.

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10

GENERAL DISCUSSION

THESIS SUMMARY

Vasopressin, the antidiuretic hormone, is important in maintaining fluid balance. In earlier experimental studies it has been shown that vasopressin has also deleterious renal effects in diabetes, and especially in autosomal dominant polycystic kidney disease (ADPKD).

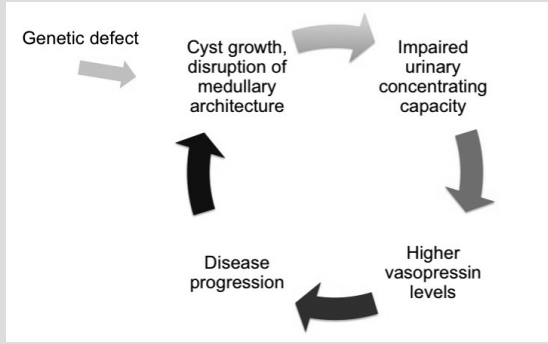
ADPKD is a genetic disease, with as most important symptom renal cyst formation and consequently kidney enlargement. This process leads to kidney failure and ultimately the need for renal replacement therapy at the median age of 55 years [1]. Vasopressin stimulates the V2 receptor in kidney collecting duct cells, which activates a pathway that has been hypothesized to lead to cyst formation and cyst growth [2]. Kidney volume has been found to be associated with the kidney function in ADPKD, but kidney volume increases before kidney function declines [3].

This thesis focuses on the role of vasopressin and kidney function in chronic kidney disease (CKD) and especially ADPKD. The first section describes the association between vasopressin, measured as copeptin, and disease progression in CKD and in ADPKD. The second section studies the effects of stimulating respectively blocking of vasopressin V2-receptors in ADPKD. In addition, the role of biomarkers indicating damage to various parts of the nephron in ADPKD is described, as well as the effect on these biomarkers during blockade of vasopressin V2-receptors in APDKD patients.

Section I. The role of vasopressin in chronic kidney disease progression.

In **chapter 2**, the alleged pathophysiological role of vasopressin in chronic kidney disease is discussed in a review. This review shows that vasopressin has deleterious renal effects in experimental as well as in human studies. There have been several mechanisms described how vasopressin can lead to renal damage: vasopressin causes hypertension and glomerular hyperfiltration by among others renin release, vasoconstriction and mesangial cell proliferation [4–6]. In this review the focus is on ADPKD, because vasopressin has a specific role in the development of cysts in this disease. Because of a genetic mutation, the polycystin complex in kidney collecting duct cells is dysfunctional, which, via a complex mechanism, leads to a rise in cAMP, which stimulates cell dedifferentiation and proliferation, and cyst fluid secretion. Importantly, patients with ADPKD have an impaired urine concentrating capacity [7], because of the destruction of normal tissue by renal cysts, which will lead to higher vasopressin levels. This gives rise to a vicious circle that may explain, at least in part, progressive renal function loss in ADPKD. This vicious circle is shown in Figure 1. In line with this hypothesis, it has been shown in animal models of ADPKD that blocking the vasopressin V2 receptor reduces cyst growth and preserves kidney function [8–11].

FIGURE 1. The vicious circle in which vasopressin leads to progressive renal function loss in ADPKD.



In **chapter 3**, the association of vasopressin and the progression of diabetic nephropathy was studied. In a cohort of 1328 diabetic patients, copeptin (a surrogate for vasopressin) was measured at baseline as well as the change in albumin/creatinine ratio and the change in kidney function (eGFR) during follow-up. This study showed that in patients not using Renin-Angiotensin-Aldosterone System (RAAS) inhibitors at baseline, copeptin was cross-sectionally associated with higher albumin/creatinine ratio and lower eGFR. During follow-up (6.5 years), copeptin was associated with an increase in albumin/creatinine ratio and a decline in eGFR. These findings support the hypothesis that vasopressin may have a pathophysiological role in the progression of diabetic nephropathy and suggest that lowering vasopressin levels might be beneficial to prevent renal damage and failure. Why there was no association between vasopressin and disease progression in patients who used RAAS inhibition remains unclear. It might be caused by a lack of power, but it can also be that the deleterious effect of vasopressin is mediated (in part) via the RAAS system. It has been suggested that vasopressin activates the RAAS system by the V1a receptor and, vise versa, that the RAAS activates the V2 receptor-Aquaporin system (12). Indeed chronic RAAS inhibition (by ACE inhibitors or Angiotensin-2 Receptor Blockers) has been found to prevent the vasopressin-induced increase in albuminuria, indicating that the RAAS mediates the effects of vasopressin [4]. More, and especially better powered research is needed to find out if vasopressin is associated with renal disease progression in diabetic patients who use RAAS inhibition, and, moreover, whether vasopressin V2 receptor antagonists will be efficacious in preventing diabetic kidney disease progression. If so, it should be investigated whether this effect will be additive to RAAS inhibition. In the chapters hereafter, we studied the role of vasopressin in specifically ADPKD.

In **chapter 4**, the association between vasopressin, measured as copeptin, and kidney function was studied in a cohort of 79 ADPKD patients with a broad range of eGFR. These patients were followed for 3 years and in this time period GFR was measured with inulin clearance. We found that the baseline copeptin level was associated with decline

in kidney function in this cohort. The same patients were followed for 11 years with GFR estimated by plasma creatinine measurements. Baseline copeptin was also associated with a decline in estimated GFR in these patients. In this cohort 9 patients started with renal replacement therapy. Eight of these 9 patients had a copeptin level at baseline that was higher than the median copeptin level. This study suggests that high vasopressin levels have a pathophysiological role in the process of renal function decline in patients with ADPKD, and that copeptin may be used as marker to identify patients at higher risk for disease progression, that may benefit of therapeutic interventions.

The above study is limited by the fact that the other important parameter for kidney disease progression in ADPKD, i.e. kidney volume, was not measured and that patients were included with a wide range in disease severity. The latter limitation precludes a conclusion whether vasopressin may have a role as early marker of worse renal prognosis. In **chapter 5**, we studied therefore the association between vasopressin (measured as copeptin) at baseline and ADPKD disease progression in 241 ADPKD patients in a relatively early stage of their disease, defined as an estimated creatinine clearance of more than 60 ml/min. Disease progression was measured as change in iothalamate assessed GFR as well as change in MRI assessed total kidney volume. In these patients copeptin level was associated with an increase in total kidney volume as well as a decrease in measured GFR during a follow-up of 8.5 years. From this study it can be concluded that vasopressin is an early marker for disease progression in ADPKD. In this study we also found that copeptin level was not associated with the expected physiological parameters, such as urine volume and plasma osmolality. This suggests that in ADPKD the normal physiology water homeostasis is disturbed.

Overall it can be concluded from Section I that vasopressin is associated with disease progression in type 2 diabetes mellitus and in ADPKD. Measuring vasopressin level might therefore be of help to predict which patients need treatment the most, because they have the highest likelihood of progressive disease. Furthermore, these data suggest that lowering vasopressin concentration or inhibiting vasopressin effects, by for instance increasing water intake, decreasing osmolar intake or by blocking the vasopressin receptor, might be beneficial in these diseases.

Section II. Blockade or stimulation of vasopressin receptors in ADPKD

Although the findings in **chapter 5** suggest that copeptin can be a marker for disease progression in patients with ADPKD in a relatively early phase of their disease, it was yet unknown whether copeptin concentration is already increased in these patients in an early stage of the disease when compared to healthy controls. In **chapter 6**, copeptin concentration was therefore investigated in well standardized circumstances, i.e. during a water deprivation test. We included 15 ADPKD patients and 15 healthy (age and gender matched) controls. All participants were asked not to drink or eat until they reached maximal urine concentrating capacity (at least 14 hours). The maximum concentrating

ability was tested using serial measurements of urine osmolality. ADPKD patients and healthy controls had a similar level of kidney function, when assessed as eGFR as well as 24hr creatinine clearance. It was found that ADPKD patients were less able to concentrate their urine, but had higher copeptin levels at the time they had reached their maximum urine osmolality. After reaching the maximum concentrating capacity, all participants received a dose of desmopressin, a vasopressin analogue, to investigate if ADPKD patients have a central (lack of vasopressin release by the hypophysis) or a nephrogenic problem (renal insensitivity for vasopressin). There was no difference in the response to desmopressin between patients and controls, making a central cause of a lower urine concentrating capacity less likely. These data suggest that kidneys of ADPKD patients are less sensitive to vasopressin and that this might be the reason why they have compensatory rise in vasopressin levels, and that this occurs already before kidney function is impaired. The cause of this impaired urine concentrating capacity is thought to be the impaired medullary osmotic gradient due to distorted renal architecture by the cyst formation in ADPKD. This is in line with the findings of an earlier study which showed that urine concentrating capacity is associated with number and size of the cysts [7]. These data also indicate that copeptin is already elevated in an early stage of ADPKD when renal function is still normal, indicating that copeptin is not elevated because of less renal clearance.

In the previous chapters it was shown that high vasopressin levels are associated with more kidney function decline and growth in total kidney volume in ADPKD. These data suggest a causal role for vasopressin in causing disease progression. Definitive evidence for this assumption has recently been provided by the results of the TEMPO 3:4 Study. In this study the use of tolvaptan, a vasopressin V2 receptor antagonist, was associated with less kidney volume increase and less function decline in ADPKD patients. In this study patients were included with a (near) normal kidney function (estimated creatinine clearance > 60 ml/min) [13]. It was therefore unknown what the renal hemodynamic effects of tolvaptan were in patients with impaired kidney function and if it was safe to use tolvaptan in these patients. In **chapter 7** the short-term renal hemodynamic effects of tolvaptan were therefore investigated in three groups of patients with ADPKD stratified for kidney function, being patients with normal kidney function (eGFR >60 mL/min/1.73m^2), moderately impaired kidney function (eGFR 30-60 mL/min/1.73m^2) and severely impaired kidney function (eGFR <30 mL/min/1.73m^2). In this study we found that GFR decreased during the use of tolvaptan in all three groups, but that this decrease was only significant in the patients with normal and moderately impaired kidney function (eGFR >30 mL/min/1.73m^2). The difference in change in GFR was only significant between patients with severely impaired kidney function (eGFR <30 mL/min/1.73m^2) and normal or moderately impaired kidney function when looking at absolute differences, not when looking at percentage change. The effective renal plasma flow did not significantly change during the use of tolvaptan in any of the three study groups. This renal hemodynamic pattern (decrease in GFR and relatively stable renal plasma flow) suggests preglomerular

vasoconstriction and a decrease in intraglomerular pressure. Such a pattern is assumed to be beneficial for preservation of kidney function on the long term. The cause of the short-term decrease in GFR during tolvaptan is not known. It might be caused by the decrease in stimulation of the vasopressin V2 receptor, which leads to a reduction of urea recycling. The tubuloglomerular feedback system is influenced by the decreased urea recycling, which will lead to a lower GFR [14]. Another cause might be that higher circulating vasopressin levels, due to the feedback mechanism by blocking the vasopressin V2 receptor, may lead to a reduction in the glomerular ultrafiltration coefficient caused by V1a receptor mediated mesangial cell contraction [15]. Importantly, these short-term changes in GFR were fully reversible after withdrawal of tolvaptan. In addition, there were no more adverse events in patients with impaired kidney function compared with patients with normal kidney function, suggesting that it is safe to use tolvaptan in patients with impaired kidney function. Of course this latter conclusion should be interpreted with caution given the relatively low number of patients that were included in this study.

In several studies urinary biomarkers were measured to predict prognosis of acute kidney injury or chronic kidney disease. In **chapter 8** biomarkers excretions were measured in urine of 102 ADPKD patients, to investigate if these biomarkers are also of use in ADPKD. We measured various urinary markers, representing different segments of the nephron. Immunoglobulin G (IgG), a large molecular size protein, which is normally not filtered in the glomerulus, is a marker for glomerular damage. NGAL, NAG and KIM-1, are markers for proximal tubular damage. HFABP is a marker for distal tubular damage, and MCP-1 and MIF are inflammatory markers. At baseline we found several associations between disease severity (measured as GFR, total kidney volume and effective renal plasma flow) and the excretion of urinary biomarkers. After this cross-sectional analysis, we followed these patients and investigated whether urinary biomarker excretion was associated with a decline in eGFR. Results of this study are given in **chapter 8b**. We found that some of our markers (albumin, IgG, KIM-1 and MCP-1), were associated with disease progression and might be useful in identifying patients who will have faster disease progression. It is surprising that IgG was found as a marker that is associated with disease progression, because this is a marker for glomerular damage. This result suggests that ADPKD is not only affecting the tubules, but that there is also a glomerular component in this disease. Higher albuminuria levels in ADPKD might therefore be caused (partly) by glomerular leakage as well, and not only by tubular damage.

The TEMPO 3:4 study showed that tolvaptan was an effective treatment for ADPKD patients with normal kidney function. An earlier experimental study by our department showed that tolvaptan is less effective in an ADPKD mouse model with impaired kidney function and suggested that tolvaptan is the most effective in an early stage of the disease [11]. In **chapter 9** the short-term effects of tolvaptan were studied in ADKPD patients with a broad range of eGFR, and the association between the effects and GFR was investigated. We looked at different short-term surrogate markers for long-term

renoprotective efficacy. During the use of tolvaptan we observed a significant increase in urine volume and plasma sodium. These changes were not dependent on GFR. Interestingly, we found that some variables like total kidney volume and urine osmolality showed less decrease in patients with lower GFR when expressed as percentage change. However, when we looked at absolute numbers, there was no significant association with baseline GFR. The most direct effect of vasopressin V2 receptor antagonists can be assessed via measurement of free water clearance, the parameter that is directly related to the physiological effect of vasopressin. We observed that free water clearance increased the most in patients with higher GFR. However, it should be taken into account that patients with impaired kidney function have a lower number of functioning nephrons. Calculation of the fractional free water clearance, i.e. free water clearance divided by GFR, adjusts for functioning nephron mass. When using this variable the efficacy of tolvaptan was the most prominent in patients with lower GFR. This suggests that tolvaptan is also effective in patients with impaired kidney function as it is in patients with normal GFR. In addition, we also observed in this study a decrease in most urinary biomarker excretions. This decrease in these biomarkers (glomerular, proximal tubular and distal tubular markers) was independent of GFR as well, suggesting that tolvaptan reduced damage to various parts of the nephron in ADPKD patients. This was found in patients with impaired kidney function as well as in patients with normal kidney function.

Overall it can be concluded from section II of this thesis that vasopressin plays an important role in ADPKD. Vasopressin is probably increased in patients with ADPKD because of their incapacity for normal urinary concentration that occurs already in an early stage of their disease, resulting in lower urine osmolality and a compensatory increase in vasopressin levels. Blocking the vasopressin V2 receptor leads to a short-term reversible GFR decrease and changes in biomarker excretion that indicate damage to various parts of the nephron, which is independent of baseline GFR. Noteworthy, tolvaptan induced changes in fractional free water clearance that were even the most prominent in patients with lower kidney function. These short-term data suggest that the efficacy of tolvaptan, a vasopressin V2 receptor antagonist, might be the same in patients with impaired kidney function compared with patients with a normal kidney function.

Future perspectives

In section I of this thesis, the association between copeptin and markers of chronic kidney disease was described in various cohorts. It seems that vasopressin plays a pathophysiological role in kidney function decline. However, all of these studies were retrospective in nature, and publication bias may have led to especially positive studies. In future, specifically designed prospective research is needed to firmly establish whether vasopressin is associated with disease progression, and whether it is vasopressin itself or other factors that influence or are influenced by vasopressin concentration and that have effects on kidney function. Such factors could be hypertension, use of antihypertensive

drugs, smoking behavior and body weight. In addition, it should also be found out whether lowering vasopressin levels will result in prevention of kidney function decline. Lowering these levels can be done by lowering plasma osmolality. Plasma osmolality is dependent of fluid intake, but also, and less well known, of osmolar intake. Therefore increasing fluid intake will result in lower vasopressin levels, but lowering the intake of sodium and protein might have the same effect. To investigate this, a randomized clinical trial is needed in which one of the groups increases fluid intake, one lowers osmolar intake, and one uses the combination of both strategies. Another way of lowering the effects of vasopressin in the kidney is by using a vasopressin V2 receptor antagonist. Tolvaptan has been shown to be effective in slowing down disease progression in ADPKD patients with a near normal kidney function [13]. In diabetes, we have found that copeptin concentration is associated with kidney function decline. Therefore it is interesting to investigate if tolvaptan could also be useful to slow down kidney function decline in diabetes. In our study we found only an association between copeptin and kidney function decline in patients not using RAAS inhibition. The cause of this difference between the two groups is unknown. It might be because vasopressin influences the RAAS system as well. Further research in patients using RAAS inhibition and lowering their vasopressin levels might elucidate the mechanism between vasopressin and the RAAS system. If this mechanism is known, it might help to find treatment options in diabetic nephropathy.

In section II of this thesis the effects of stimulating and blocking vasopressin activity was investigated. We found that in ADPKD patients, vasopressin is already elevated in an early stage of the disease, probably because of insensitivity for vasopressin of the kidneys. Blocking the vasopressin V2 receptor resulted in a reversible decrease in GFR in patients with ADPKD. This decrease was lower in patients with impaired kidney function. In addition, we found a similar number of adverse events in patients with impaired kidney function, when compared to patients with normal kidney function. Therefore it seems safe to use tolvaptan in these patients, but more research is needed to definitively prove the safety of this drug in patients with impaired kidney function. Especially long term data are needed in a large cohort of ADPKD patients.

Because the effect on GFR was less in patients with impaired kidney function, it might be that these patients have less benefit of tolvaptan. We therefore investigated in the same study the effects of short-term tolvaptan treatment on parameters that may be surrogates for long-term renoprotection, and the association of these effects with baseline GFR. Some of these variables showed less absolute response on tolvaptan in patients with impaired kidney function. However, when looking at relative changes, treatment response was not related to baseline GFR. Fractional free water clearance, i.e. free water clearance indexed for GFR, might be the most direct measure for vasopressin activity. Interestingly, free water clearance increased most in patients with higher GFR. However, the fractional free water clearance increased most in patients with lower GFR. These divergent results show that it is difficult to draw a firm conclusion on the efficacy

of tolvaptan in patients with impaired kidney function. Long-term studies using “hard” end-points are therefore needed to firmly establish the potential renoprotective effects of tolvaptan in ADPKD patients with impaired kidney function.

The adverse events of tolvaptan that have been reported are mainly thirst, polyuria and nocturia. These adverse events may limit the feasibility of life-long treatment with this drug. From earlier studies it is known that most of the adverse events are dose dependent [16]. Therefore, it might be easier to tolerate tolvaptan when dosages are lower. Since this may be expected to result in less renoprotection, the drug should then be combined with other medications that slow down disease progression to have the same efficacy. An additional problem with the use of this drug is that liver cysts, another possible symptom of ADPKD, are probably not influenced by vasopressin V2 receptor antagonists, because liver tissue lacks these V2 receptors. For these reasons it is necessary to develop other effective treatment strategies. There are several pathophysiological pathways that theoretically influence cyst growth in ADPKD and that are open for therapeutic interventions [1]. Several studies were for instance performed investigating mTOR inhibition. Unfortunately, these studies were negative on the long-term [17, 18]. Another possibility is the somatostatin pathway. In polycystic liver disease it has been shown that lanreotide, a somatostatin analogue, decreased the increase of liver cysts, and possible also led to less growth in kidney volume [19]. These studies were, however, of short duration (6-12 months) and included only relatively small numbers of patients, which does not allow firm conclusions on the possible renoprotective effects of these drugs. A new trial has recently started in the Netherlands to investigate the effect of lanreotide on kidney cysts and kidney function (the DIPAK-1 study) [20]. This is a well-powered multi-center randomized controlled clinical trial, including 300 patients with 30 months of follow-up. Endpoints are kidney function and kidney volume. If lanreotide is effective in this trial, patients who cannot tolerate tolvaptan or can only tolerate low doses, might want to use lanreotide instead or as combination therapy with tolvaptan.

ADPKD is a disease showing considerable variety in disease severity and rate of disease progression between patients, even when patients share the same mutation [3]. There are patients that never reach end-stage kidney failure, but there are also patients that need renal replacement therapy already in the fourth decade of their life. In one of the studies described in this thesis, various biomarkers were measured in urine of ADPKD patients and most of them were associated with disease severity. The biomarkers indicate damage to different segments of the nephron. To our surprise, not only tubular markers, but also glomerular and inflammatory markers were associated with disease severity. During follow-up we found that some of these markers were independently associated with the rate of disease progression in ADPKD during long-term follow-up. This suggests that it may be useful to measure these markers to identify patients with a high likelihood of rapid disease progression and that need treatment to prevent this. Measuring these biomarkers may also be helpful to identify patients that do not need treatment, with the advantage

that these patients will not have to face the adverse events treatment will have, and, moreover, it will save costs. In another study we have seen that the excretion of urinary biomarkers was lowered during the use of tolvaptan, suggesting that these markers may also be useful as short-term surrogate markers to indicate potential long-term treatment efficacy. In case future studies will show that the patients with the most short-term decrease in biomarker excretion are indeed the ones who have the most benefit from long-term treatment, these biomarkers can be used to find out which treatment option will be the best for a particular patient, and may even be of help to titrate the dosage of such treatment.

In conclusion, these are exciting times in the research field of ADPKD. The first effective drug treatment to slow down disease progression in ADPKD has recently been found, research is ongoing to develop other treatment options and to establish biomarkers that can predict prognosis and help to titrate treatment. A once untreatable disease seems to become treatable.

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NEDERLANDSTALIGE SAMENVATTING

VASOPRESSINE

Vasopressine, ook wel antidiuretisch hormoon genoemd, is een hormoon dat er voor zorgt dat de waterhuishouding in het menselijk lichaam in balans blijft. Wanneer het bloedvolume in het lichaam laag wordt of de concentratie zout in het bloed te hoog, wordt er meer vasopressine uitgescheiden vanuit de hypofyse (een klier bij de hersenen) naar het bloed om zo meer water terug te halen uit de voorurine. Vasopressine bindt aan de V2-receptoren (deel van de cel waar specifieke stof aan kan binden, wat een reactie geeft in de cel) in de nier, waardoor de urine geconcentreerder wordt. Wanneer men veel water drinkt, wordt er minder vasopressine uitgescheiden, en wordt de urine minder geconcentreerd; men plast meer water uit en blijft zo in balans.

Behalve de V2-receptoren bindt vasopressine ook aan V1a- en V1b-receptoren. Binding aan V1a-receptoren leidt tot vasoconstrictie (vernauwen van bloedvaten door de daar aanwezige spieren), glucoseaanmaak door de lever, vrijkomen van verschillende stollingsfactoren (VII en de von Willebrandfactor) en makkelijker samenklonteren van bloedplaatjes. Binding aan V1b leidt tot vrijkomen van corticotropine, een hormoon dat onder andere het vrijkomen van cortisol, het stresshormoon, stimuleert.

Vasopressine is niet gemakkelijk te bepalen in bloed, doordat het gedeeltelijk gebonden is aan bloedplaatjes en het buiten het lichaam snel afbreekt. Vasopressine wordt als onderdeel van een groter eiwit vrijgelaten door de hypofyse. Een ander onderdeel van dit grotere eiwit is copeptin. Copeptin is gemakkelijker te meten in het bloed, doordat dit wel stabiel is buiten het lichaam en niet gebonden is aan bloedplaatjes. Copeptin komt in gelijke mate vrij met vasopressine en is daarom een goede marker om te bepalen hoeveel vasopressine er vrijgekomen is.

DEEL I DE ROL VAN VASOPRESSINE IN PROGRESSIE VAN CHRONISCHE NIERZIEKTE.

In meerdere dierexperimentele studies is bewezen dat verhoogde concentraties vasopressine in het bloed leidt tot hypertensie (hoge bloeddruk) en verhoogde filtratiesnelheid in de nier. Dit leidt op de langere termijn tot verlittekening van de glomerulus (de filter van de nier) en eiwitverlies in de urine. Wanneer de receptor voor vasopressine geblokkeerd wordt in de nier, leidt dit tot minder eiwitverlies in de urine en voorkomt verlittekening van de glomerulus. In een studie bij mensen bleek dat het toedienen van vasopressine per infuus ook leidde tot eiwitverlies in de urine. Daarnaast is in een grote database met gegevens van de algemene bevolking gebleken dat mensen met hogere copeptinwaardes meer eiwitverlies in de urine hadden. Ook is gebleken dat hogere copeptinwaardes bij patiënten die een donornier hadden ontvangen geassocieerd was met meer nierfunctieachteruitgang bij deze patiënten. Al met al was er dus al enig bewijs dat vasopressine een negatief effect had op de nieren. In **hoofdstuk 2** wordt een overzicht gegeven van de bestaande literatuur over de rol van vasopressine in chronische nierziekten.

In **hoofdstuk 3** wordt een onderzoek naar copeptin en de relatie met nierfunctieachteruitgang en een toename van eiwitverlies in de urine bij patiënten met diabetes (suikerziekte) beschreven. Het was al bekend dat bij diabetespatiënten hogere concentraties copeptin werden gemeten dan bij gezonde mensen en dat gezonde mensen met hoge concentraties copeptin vaker diabetes kregen. De vraag of copeptinconcentratie ook samenhing met nierfunctieachteruitgang bij diabetespatiënten was nog niet beantwoord en dit werd daarom onderzocht in **hoofdstuk 3**. Er werd bij de 1328 deelnemers aan de ZODIAC-studie (een grote studie bij diabetespatiënten in de regio Zwolle) copeptin gemeten in het bloed dat was afgenomen aan het begin van de studie en vervolgens werd de nierfunctie en eiwitverlies in de urine vervolgd. Aan het begin van de studie was copeptin geassocieerd met een hoger eiwitverlies en een lagere nierfunctie. Gemiddeld werden deze patiënten 6,5 jaar gevolgd en het bleek dat bij patiënten die geen medicatie hadden die invloed had op het RAAS-systeem (renine-angiotensine-aldosteron systeem, een systeem dat invloed heeft op de bloeddruk), copeptin geassocieerd was met nierfunctieachteruitgang. Copeptin was zelfs sterker geassocieerd met nierfunctieachteruitgang dan de bekende risicofactoren zoals overgewicht en bloeddruk. Dit resultaat wekt de suggestie dat verlaging van het vasopressinegehalte in het bloed zou kunnen leiden tot het voorkomen van nierfalen bij diabetespatiënten. Om dit te onderzoeken is een nieuwe studie nodig waarin dit hormoon geblokkeerd wordt door bijvoorbeeld vasopressine-receptor blokkers zoals tolvaptan of door het verlagen van dit hormoon door bijvoorbeeld veel water te drinken.

Autosomaal dominante polycysteuze nierziekte (ADPKD)

Hoofdstuk 4 gaat over de associatie tussen copeptin en nierfunctie in patiënten met ADPKD. ADPKD staat voor “autosomal dominant polycystic kidney disease”, oftewel de erfelijke aandoening cystenieren. Dit is een aandoening die bij 1 op de 400 tot 1000 mensen voorkomt en die leidt tot vorming van cystes (holtes gevuld met vocht) in de nieren. De mogelijke symptomen zijn pijn (in de flanken), bloed bij de urine, hoge bloeddruk en een afname van nierfunctie gedurende de jaren. Daarnaast kunnen er ook nog buiten de nieren symptomen zijn van de ziekte zoals levercystes, hartklepafwijkingen en aneurysmata (vaatverwijdingen) in de hersenvaten. De cystes in de nieren groeien gedurende het leven en daardoor neemt de niergrootte steeds verder toe. De nierfunctie blijft echter nog lang behouden ondanks dat de nieren groeien. Het aantal cystes en de niergrootte is echter wel gerelateerd aan achteruitgang in nierfunctie en niergrootte is dan ook een vroege voorspeller voor nierfunctieachteruitgang.

ADPKD wordt veroorzaakt door een mutatie in het PKD1 of PKD2 gen. Door deze mutatie is het polycystine-eiwit afwijkend. Door deze afwijking reageren de cellen in de tubuli (nierbuisjes) afwijkend op bepaalde stimuli en ontstaan er cystes in de nier. Het ontstaan en groeien van deze cystes wordt via een ingewikkeld reactiepad waarschijnlijk onder andere gestimuleerd door de binding van vasopressine aan de V2-receptoren.

Uit eerder onderzoek was al gebleken dat de concentratie copeptin (als marker voor vasopressine) geassocieerd was met grotere nieren en lagere nierfunctie bij ADPKD-patiënten. Door deze relatie verwachtten we dat copeptin ook als voorspellende marker gebruikt zou kunnen worden.

In **hoofdstuk 4** wordt een onderzoek beschreven naar copeptin en ADPKD. Er werd bloed afgenomen aan het begin van een studie waar 79 ADPKD-patiënten aan mee deden. In dit bloed werd copeptin gemeten. Patiënten werden in die studie 3 jaar gevolgd, waarbij op een nauwkeurige manier de nierfunctie jaarlijks gemeten werd (inulineklaring) gedurende gemiddeld 3,3 jaren. Daarnaast werd de geschatte nierfunctie middels het kreatinine in het bloed gedurende gemiddeld 11,2 jaren gevolgd. De copeptinconcentratie aan het begin van de studie bleek significant geassocieerd te zijn met nierfunctieachteruitgang in beide meetmethodes. Daarnaast waren er gedurende de 11 jaren van follow-up 9 mensen getransplanteerd of met dialyse begonnen. Van deze 9 patiënten hadden 8 een bovengemiddelde copeptinconcentratie in het bloed. Deze studie suggereert met deze uitkomsten dat vasopressine inderdaad een rol speelt in de achteruitgang van nierfunctie bij ADPKD-patiënten. Deze studie heeft als nadeel dat er een relatief kleine groep patiënten met een erg brede range aan nierfunctie meededen, nierfunctie geschat werd en niet nauwkeurig gemeten en er geen niergrootte is bepaald bij deze patiënten.

In **hoofdstuk 5** is er opnieuw gekeken naar de relatie copeptin en progressie van ziekte bij ADPKD, maar dan niet alleen naar nierfunctie, maar ook naar niergrootte. Niergrootte is een vroegere marker van ernst van ziekte dan nierfunctie. Voor deze studie werd gebruik gemaakt van de CRISP-database, een database met gegevens van 241 ADPKD patiënten in de Verenigde Staten. In deze studie werd er in het bloed, afgenomen op de eerste dag van de studie, copeptin bepaald en deze patiënten werden gemiddeld 8,5 jaren gevolgd met metingen van niergrootte (middels MRI) en nauwkeurig gemeten nierfunctie (middels iothalamaatklaring). Om aan deze studie te mogen deelnemen, moesten de patiënten een redelijk goede nierfunctie hebben (kreatinineklaring >60 ml/min). In deze studie was copeptinconcentratie inderdaad geassocieerd met ziekteprogressie.

In het algemeen kan uit deel 1 van dit proefschrift geconcludeerd worden dat copeptin, als marker voor vasopressine, is geassocieerd met ziekteprogressie bij diabetes en ADPKD. Copeptin meten kan daarom helpen met het selecteren van patiënten met een verwachte ernstiger beloop van de ziekte. Verder suggereren deze resultaten ook dat het verlagen van vasopressineconcentraties in het bloed achteruitgang zou kunnen voorkomen.

DEEL 2 BLOKKADE OF STIMULATIE VAN VASOPRESSINERECEPTOREN IN ADPKD

In dit deel van het proefschrift worden de effecten van stimulatie (door een dorstproef) en blokkade (door een medicijn dat de V2 receptor blokkeert) van vasopressine receptoren in de nier beschreven bij patiënten met ADPKD. Hoewel bleek uit **hoofdstuk 5** dat

copeptin mogelijk een marker is voor ziekteprogressie bij ADPKD in een vroeg stadium van de ziekte, was het nog niet duidelijk of de copeptinconcentratie al verhoogd was bij ADPKD-patiënten in een vroege fase van de ziekte in vergelijking met gezonde mensen. In **hoofdstuk 6** werden daarom copeptinconcentraties in het bloed van 15 patiënten met ADKPD en nog een goede nierfunctie vergeleken met de concentraties copeptin in 15 gezonde mensen (gematcht met de patiënten op leeftijd en geslacht). Dit werd gedaan onder gestandaardiseerde omstandigheden, namelijk in een dorstproef. Deelnemers werden gevraagd niet te eten en te drinken gedurende 14 uren, daarna werd er ieder uur naar de osmolaliteit van de urine gekeken (een maat voor hoe geconcentreerd de urine is) totdat de urine de maximale osmolaliteit bereikte (twee keer achter elkaar een gelijke osmolaliteit). Het was uit oude literatuur al bekend dat ADPKD-patiënten hun urine minder goed kunnen concentreren dan gezonde mensen, waarschijnlijk door de cystes die de normale structuur van de nier verstoren. Uit dit onderzoek is gebleken dat patiënten met ADPKD al in een vroege fase van de ziekte, namelijk op het moment dat er nog een normale nierfunctie is, al minder goed hun urine kunnen concentreren. Daarnaast hadden ze op dat moment ook een hogere copeptinconcentratie in het bloed in vergelijking met de gezonde controles. Waarschijnlijk is er een vicieuze cirkel gaande: doordat de ADPKD patiënten hun urine niet goed kunnen concentreren, wordt door een feedbackmechanisme hun vasopressineconcentratie (en dus copeptin) verhoogd. Echter, de nieren kunnen niet beter concentreren, waardoor de urine osmolaliteit niet verder stijgt en de vasopressineconcentratie verder verhoogd wordt. Op deze manier hebben ADPKD-patiënten een sterk verhoogde concentratie vasopressine (en dus copeptin) in het bloed, zonder dat de urine geconcentreerder raakt. Er werd ook nog getest of patiënten met ADPKD wel voldoende vasopressine aanmaakten, wat eventueel ook een oorzaak zou kunnen zijn voor verminderd concentrerend vermogen van de urine. Na een injectie van een soort vasopressine werd het concentrerend vermogen niet verbeterd, waardoor het probleem niet in de aanmaak in de hersenen lijkt te liggen, maar in de reactie van de nieren.

In deel 1 van dit proefschrift werd een verband gevonden tussen vasopressine (gemeten als copeptin) en nierfunctieverslechtering bij ADPKD. Het bewijs dat het blokkeren van vasopressinereceptor in de nier, door middel van tolvaptan, een vasopressine-receptor antagonist, een gunstig effect heeft in ADPKD, werd recent geleverd. De TEMPO-studie was een wereldwijde studie naar tolvaptan bij patiënten met ADPKD. Er werden 1445 patiënten geïncludeerd met een nog goede nierfunctie (geschatte kreatinineklaring >60 ml/min) voor de studie en zij werden drie jaar lang gevolgd. In deze geblindeerde studie kreeg een derde van de patiënten een placebo, twee derde kreeg tolvaptan. Uit deze studie bleek inderdaad dat patiënten die behandeld werden met tolvaptan minder toename van niervolume en minder afname van nierfunctie hadden.

Omdat alleen patiënten met een goede nierfunctie mochten deelnemen, bleef het onduidelijk wat het effect van tolvaptan op filtratiesnelheid en doorbloeding van de nieren

was bij patiënten met een verminderde nierfunctie. In **hoofdstuk 7** werd daarom een studie verricht waarbij 9 patiënten met een goede nierfunctie (geschatte filtratiesnelheid >60 ml/min), 9 patiënten met een matig gestoorde nierfunctie (30-60 ml/min) en 9 patiënten met een verlaagde nierfunctie (<30 ml/min) werden geïncludeerd. Bij hen werd een nauwkeurige nierfunctiemeting verricht (zowel filtratiesnelheid (glomerular fitration rate, GFR) als doorbloeding (effective renal plasma flow, ERPF)) voor de start met tolvaptanbehandeling, op de 21e dag van behandeling en 3 weken na staken van de behandeling. Daarnaast werden ook bloeddruk, bloedwaardes (bijvoorbeeld natrium), gewicht en bijwerkingen bijgehouden. Al deze waardes werden vergeleken tussen de drie onderzoeksgroepen, om te zien of de effecten van tolvaptan anders waren bij patiënten met een verminderde nierfunctie in vergelijking met patiënten met een goede nierfunctie. De filtratiesnelheid (GFR) verminderde in alle drie groepen tijdens het gebruik van tolvaptan, maar deze afname was alleen significant bij patiënten met een filtratiesnelheid hoger dan 30 ml/min. Na het staken van de behandeling, was de nierfunctie niet anders dan voor behandeling. De doorbloeding veranderde niet significant tijdens tolvaptangebruik. Dit patroon van veranderingen (verlaagde GFR en gelijke ERPF) past bij een verlaging van bloeddruk in de glomerulus (de nierfilter). Verlaging van deze druk zorgt in het algemeen voor verminderde schade op de lange termijn. In dit onderzoek was er geen verschil in aantal bijwerkingen tussen de verschillende groepen. Al met al leek er geen nadelig verschil te zijn voor de patiënten met een lagere nierfunctie in vergelijking met patiënten met betere nierfuncties in de effecten op filtratiesnelheid, doorbloeding of bijwerkingen. De meest voorkomende bijwerkingen waren: dorst, droge mond, vaak moeten plassen en 's nachts moeten plassen. Dit zijn verwachte bijwerkingen, aangezien het blokkeren van de receptor voor vasopressine leidt tot meer wateruitscheiding en daardoor tot vaker plassen en het krijgen van dorst.

Om de effectiviteit te meten op ziekteprogressie bij ADPKD wordt er meestal gebruik gemaakt van nierfunctie (GFR) en niervolume (grootte van de nieren). Deze variabelen zijn niet goedkoop of gemakkelijk nauwkeurig te meten. Het zou daarom interessant zijn om goedkopere en makkelijker te meten variabelen te hebben die ook de achteruitgang in nierfunctie kunnen voorspellen. In **hoofdstuk 8** werden daarom biomarkers in de urine gemeten. Deze biomarkers komen in de urine bij schade van verschillende onderdelen van de nier. In urine van 24 uur van 102 ADPKD-patiënten en 102 op leeftijd en geslacht gematchte gezonde deelnemers werd de totale uitscheiding van verschillende markers gemeten en er werd onderzocht of deze uitscheiding geassocieerd waren met GFR, ERPF en niergrootte bij patiënten met ADPKD. De uitscheiding van alle biomarkers was verhoogd in patiënten met ADPKD in vergelijking met gezonde controles. Er waren meerdere biomarkers geassocieerd met de verschillende maten van ernst van ziekte, waarbij NGAL (neutrophil gelatinase-associated lipocalin), een marker voor het bovenste gedeelte van de tubulus (nierbuisje), met zowel nierfunctie als niergrootte geassocieerd was. Van de patiënten die daarna niet deel hadden genomen aan een interventiestudie

maar wel gevolgd werden (46 patiënten), kon de associatie bekeken worden tussen de hoogte van biomarkeruitscheiding aan het begin en de afname van geschatte nierfunctie (estimated GFR, eGFR) in de periode dat ze gevolgd werden. Hieruit bleek dat er 4 biomarkers significant geassocieerd waren met nierfunctieachteruitgang. Deze markers lijken daarom gebruikt te kunnen worden om ziekte-ernst te meten en mogelijk zouden ze ook gebruikt kunnen worden om de patiënten te selecteren die snel achteruitgaan of om effectiviteit van behandeling te meten.

In de studie die beschreven is in **hoofdstuk 7** werden de effecten van tolvaptan op doorbloeding, filtratie en bijwerkingen beschreven. In dit onderzoek is ook gekeken naar de associatie tussen nierfunctie en (korte-termijn) effectiviteit van tolvaptan. De resultaten staan beschreven in **hoofdstuk 9**. In een eerdere experimentele studie met diermodellen, werd gesuggereerd dat tolvaptan minder goed werkte in een later stadium van ADPKD. Daarom werd er in de kleine groep patiënten met een behandeling van 3 weken gekeken naar de effectiviteit van tolvaptan. Effectiviteit werd op verschillende manieren gemeten, zoals verandering in niergrootte, hoeveelheid urine, vrije waterklaring en de uitscheiding van biomarkers zoals beschreven in **hoofdstuk 8**. Tijdens het gebruik van tolvaptan nam de hoeveelheid urine per 24 uur sterk toe en ook het natriumgehalte in het bloed was significant verhoogd bij alle patiënten. Niergrootte en osmolaliteit (geconcentreerdheid van urine) namen percentueel minder af bij patiënten met een lagere nierfunctie. Wanneer echter niet naar percentage maar naar absolute getallen gekeken werd, was er geen verschil in verandering van deze getallen tussen patiënten met goede en verminderde nierfunctie. Wanneer de vasopressine V2-receptor geblokkeerd wordt in de nier door tolvaptan, gaat de nier meer water uitscheiden zonder andere osmolen, vrij water genoemd. De hoeveelheid vrije waterklaring kan daarom gebruikt worden om de reactie op tolvaptan te meten. Vrije waterklaring nam het meest toe in patiënten met een betere nierfunctie. Echter, patiënten met een minder goede nierfunctie, hebben ook minder werkende nefronen (onder andere nierfilters). Wanneer de vrije waterklaring wordt gedeeld door de GFR (filtratiesnelheid, een maat voor hoeveelheid werkende nierfilters), dan is de toename juist het grootst bij patiënten met de slechtste nierfunctie. In deze studie werden ook de biomarkeruitscheiding in urine gemeten. De afname in uitscheiding van deze biomarkers was onafhankelijk van nierfunctie en de afname was aanwezig in biomarkers die schade van verschillende delen van de nier weergeven, suggererend dat tolvaptan de schade in meerdere delen van de nier verlaagt. Concluderend wekt deze studie de suggestie, dat tolvaptan even goed werkzaam is bij patiënten met een verminderde nierfunctie. Een grotere studie is nodig om hier meer over te kunnen zeggen.

Samenvattend kan er uit deel 2 van dit proefschrift de conclusie getrokken worden dat vasopressine een belangrijke rol speelt in ADPKD. Het is al in een vroeg stadium verhoogd door de verminderde capaciteit om urine te kunnen concentreren. Het blokkeren van de receptor voor vasopressine in de nier leidt tot een omkeerbare afname van GFR en een afname van schademarker-uitscheiding onafhankelijk van nierfunctie. Effectiviteit

van tolvaptan lijkt dus gelijk te zijn bij patiënten met een verminderde nierfunctie in vergelijking met patiënten met een normale nierfunctie.

TOEKOMSTPERSPECTIEVEN

In deel 1 van dit proefschrift werd de associatie tussen copeptin en markers voor chronische nierziekte beschreven. Helaas zijn al deze studies retrospectief, en verschillende soorten van bias (factoren die de betrouwbaarheid van data kunnen beïnvloeden) zouden hier invloed op gehad kunnen hebben. In de toekomst zou het daarom goed zijn om een studie op te zetten waarbij er op baseline copeptin (en eventueel vasopressine) gemeten wordt en de patiënten te volgen. Daarnaast zouden ook factoren, die vasopressine beïnvloeden of door vasopressine beïnvloed worden, gemeten moeten worden (bijvoorbeeld bloeddruk, medicatie, roken, lichaamsgewicht, zout- en eiwitinname). Er zou dan ook een studie gedaan kunnen worden om te zien of verlaging van vasopressine inderdaad nierfunctieachteruitgang kan verminderen. Het verminderen van vasopressine kan bereikt worden door de osmolaliteit van bloed te verminderen door bijvoorbeeld meer water te drinken of minder osmolen in te nemen (minder zout en minder eiwit). Om deze studie uit te voeren is een gerandomiseerde studie nodig waarbij een groep meer water drinkt, een groep minder osmolen inneemt en een groep die beide combineert in vergelijking met een groep patiënten die niets verandert aan het dieet. Een andere manier om het effect van vasopressine te beïnvloeden is door middel van een receptorblokker, zoals tolvaptan. Hiermee zijn al verschillende studies gedaan bij ADPKD zoals beschreven in dit proefschrift, maar nog niet bij diabetespatiënten. Het zou interessant zijn om te onderzoeken of tolvaptan ook nierfunctieachteruitgang kan voorkomen bij diabetespatiënten.

In deel 2 van dit proefschrift werd onder andere onderzocht wat de effecten waren van tolvaptan bij ADPKD patiënten met een verminderde nierfunctie. De uitkomst was dat de effecten bij patiënten met een verminderde nierfunctie waarschijnlijk niet verminderd is, maar dit is getest in een kleine groep patiënten met 3 weken behandeling. Daarnaast leek de veiligheid ook niet verminderd, maar dit kan alleen geconcludeerd worden indien er een grotere en langere studie zal plaatsvinden bij ADPKD-patiënten met een verminderde nierfunctie.

De bijwerkingen van tolvaptan zijn vooral dorst, veel moeten plassen en 's nachts moeten plassen. Door deze bijwerkingen is het waarschijnlijk moeilijk om levenslang dit medicijn te gebruiken. De bijwerkingen zijn afhankelijk van de dosis die gebruikt wordt, maar de effectiviteit is ook afhankelijk van de dosis. Een lagere dosis tolvaptan in combinatie met een ander medicijn, dat ook nierfunctieachteruitgang vermindert, zou daarom mogelijk makkelijker te verdragen zijn maar toch dezelfde effectiviteit hebben. Behalve de bijwerkingen, heeft tolvaptan ook het nadeel dat het de vasopressinereceptoren in de nier blokkeert, maar daardoor niet werkt op levercystes. Er zijn meerdere theoretische

manieren om de cystegroei in nier en lever te beïnvloeden. Een daarvan is het remmen van de zogeheten mTOR-route. Helaas komen er uit de studies naar medicatie die deze route remmen geen positieve resultaten op de lange termijn. Een andere manier is het stimuleren van de somatostatine-route. Bij polycysteuze leverziekte is gebleken dat lanreotide (via de somatostatine-route) de groei van levercystes remt en mogelijk ook de groei van niercystes. De studies hiernaar waren echter kort (6-12 maanden) en getest in een klein aantal patiënten. Recent is een nieuwe studie gestart in Nederland waarin de effectiviteit van lanreotide op niercystes en nierfunctie wordt onderzocht. Dit is de DIPAK-I studie, een studie die in meerdere centra in Nederland plaatsvindt en waaraan totaal ongeveer 300 patiënten zullen deelnemen. Deze patiënten zullen in twee groepen verdeeld worden (wel of geen behandeling) en 30 maanden gevolgd worden. Indien lanreotide ook effectief blijkt te zijn bij ADPKD, zou dit misschien een medicijn kunnen zijn wat een alternatief biedt voor tolvaptan of in lage dosis gecombineerd kan worden met lage dosis tolvaptan.

ADPKD is een aandoening met een grote variatie van ernst en snelheid van ziekteprogressie. Sommige patiënten hoeven nooit in dialyse of transplantatie, terwijl er ook patiënten zijn die dialyse of transplantatie nodig hebben voor hun 40e levensjaar. In dit proefschrift werd een studie beschreven waarin biomarkers gemeten werden die schade aangeven in de nier. Uit deze studie bleek dat de schademarkers, zowel markers van de tubulus (nierbuisjes, de plaats waar de cysten ontstaan) als van de glomerulus (nierfilter), goed correleerden met de ziekte-ernst. Indien de hoogte van de schademarkers kunnen voorspellen wat de snelheid van nierfunctieachteruitgang is bij een ADPKD-patiënt, zou middels deze markers een selectie gemaakt kunnen worden tussen patiënten die wel en die geen behandeling nodig hebben om transplantatie of dialyse te voorkomen. Daarnaast zouden de biomarkers mogelijk gebruikt kunnen worden om de optimale dosering van behandeling te kunnen bepalen per patiënt.

Er is op dit moment dus veel gaande in het onderzoeksveld op het terrein van ADPKD. De eerste effectieve behandeling is gevonden om ziekteprogressie af te remmen en er lopen meerdere onderzoeken naar andere behandelopties en biomarkers die de prognose van de ziekte kunnen voorspellen. Al met al lijkt een ziekte die onbehandelbaar was toch behandelbaar te worden.

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